

Time for Elitism

A CONGRESS of barbers, gathered to hear the latest about hairdressing techniques and told by their president that in the past thirty years the quality of their work had declined, that they were taking too long to cut customers' hair and that they overcharged the public, would undoubtedly be in a lynching mood. The barbers, exposed for what they were, on returning to their shops would find an angry public demanding refunds. For this reason, if no other, senior members of trades and professions are not given to criticizing their colleagues in public—except, that is, for Professor Kenneth Mellanby.

At the annual meeting of the British Association this year Professor Mellanby, President of Section X and Director of the Nature Conservancy Monks Wood Experimental Station, announced that science was overstaffed and mismanaged. He said that although funding has multiplied a hundred-fold since the 1930s "productivity", however determined, has declined sadly. More money has meant more jobs, and the larger the "team", the less the creative mind around which this structure is developed can do anything but administrate. The only corrective Professor Mellanby sees is an unashamedly elitist approach—support the good and do not allow the less than good to pretend they are doing constructive research.

Two issues need discussion. One is simply whether Mellanby is making a correct judgment. The other is that if he is indeed right, why does not a statement like this raise more than a ripple?

The theme that science is not what it used to be is not a new one. As long ago as 1830, Charles Babbage could write a book persuasively and wittily on unfavourable trends, including expansionism, among scientists. He would have had no difficulty in revising it for the 1970s. The present day problem seems to be a lack of incisiveness among scientists in converting personal opinions into action. Although it is indisputable that the contributions of some scientists are consistently brilliant and the contributions of others are consistently negligible, the processes of natural selection are seriously impaired by some form of gentlemen's agreement which classifies open criticism of the mediocre as intolerable rudeness. The process starts early when failure to get a PhD is seen as such a humiliation both for student and adviser that the standards of the degree are seriously depressed to accommodate students who are not natural research workers.

The fault is not the student's because no one has had the courage to say "you are not going to make the grade". Then, armed with a certificate to do research, most will settle for nothing less, and none will say nay because who can tell what someone might come up with?

This is a serious misreading of the character of observational science. No one visiting Cambridge should fail to observe Eric Gill's stone inscription on the Department of Zoology *Dans les champs de l'observation, le hasard ne favorise que les esprits préparés*: scientists of distinction lead a favoured life, not because they are lucky but because they know how to capitalize on their luck. To encourage those to stay in the field who clearly lack the ability out of a hope that something may turn up is often

no more profitable than providing monkeys with typewriters because they might turn out a work of genius.

The overpopulated ranks of research are not, however, merely an expensive phenomenon. They do, as Mellanby points out, constrict, if not throttle, the creativity of the bright who frequently lack the political skill or authority to thin out teams of research workers willed on them. The administrative load can be crippling, of course, although it is frequently self-imposed, but there is also the intellectual load of devising thesis topics and trying to keep employees abreast of original research.

All this suggests considerable reduction in the ranks of those who call themselves researchers. It is not easy to suggest means of doing this which do not hurt, but in trying to give an answer to the question "Why a mere ripple from this statement?", the urgency of finding an answer may at least be underlined.

Until now there has been little feeling amongst scientists that they are living largely off taxpayers' money (many industrial scientists included), nor does the taxpayer readily associate criticism of scientific research with his tax bill. Yet the amount that he pays in Britain for the research council (a modest fraction of the total research and development expenditure) is at least as much as he pays each year for having his hair cut. Awareness of this (perhaps not in such specific terms) cannot but increase—the Apollo space programme, if it did nothing else for the man in the street in the United States, gave him an insight into the relationship between taxes and research spending.

It is arguable that Britain is beginning to become a post-industrial society. If so, science will not occupy the revered position it has for the past hundred years and we will hear more of the market-place approach to expenditure on research—if composers, printers, poets and sportsmen have to survive on the quality of what they provide, should research scientists be exempt?

100 Years Ago



AMONG Messrs. [Macmillan's announcements of forthcoming works are—"On the Theory of Sound," by Lord Rayleigh, F.R.S.; "Contributions to Solar Physics," by J. Norman Lockyer, F.R.S., with numerous illustrations; "Cave Hunting," by W. Boyd Dawkins, F.R.S., being researches on the evidence of caves respecting the early inhabitants of Europe; "The Origin and Metamorphoses of Insects," by Sir John Lubbock, F.R.S. (vol. ii. NATURE Series); and a new edition of Canon Kingsley's "Glaucus."

From *Nature* 8, 390, September 11, 1873



Sakharov Taken to Task in *Pravda*

IN July Dr Andrei Sakharov, the Soviet nuclear physicist, gave a widely publicized interview to Swedish television. He vigorously attacked a system perpetuating criminality and privilege and pointed to favoured treatment that was afforded to Communist Party members in medical care. The Academician also denounced lack of freedom both in ideas and travel. The literature, he said, is "grey, official, solemn and boring"; travel is restricted both within the Soviet Union and beyond so that Soviet society is divided by visa constraints and Soviets are not afforded sufficient opportunities to leave the country and return.

Since then Dr Sakharov has been warned by the Deputy General Procurator about his civil rights activities,

and in particular this interview. He says that he was told the Procuracy could no longer stand aside, as these activities were providing information of interest to foreign intelligence services.

In the past few days several letters have been published in *Pravda* concerning Sakharov's activities—from, amongst others, members of the Academy of Agricultural Sciences, the Ukrainian Academy of Sciences, the Siberian branch of the Academy of Sciences and a lathe operator in Leningrad. We reprint translations of two of the most interesting letters, from members of the Soviet Academy of Sciences and from a group of distinguished Soviet composers.

We feel it necessary to bring to the attention of a wide public our attitude to the behaviour of Academician A. D. Sakharov.

In recent years, Academician Sakharov has turned away from active scientific work and has come out with a number of statements discrediting the state system and the external and internal policies of the Soviet Union. Recently, in an interview to foreign correspondents in Moscow which was published in the Western press, he reached the stage where he came out against the policy of the Soviet Union of relieving international tension and of consolidating those positive changes which have taken place recently throughout the world.

These statements, which are completely alien to the interests of all progressive peoples, Sakharov tries to justify by crude distortions of Soviet activity and imaginary reproaches regarding the socialist system. In his dicta he in effect takes up a position of solidarity with the most reactionary imperialist circles, coming out actively against the course of the peaceful coexistence of countries with different social systems, against our party and state attitudes to the development of scientific and cultural cooperation and to the strengthening of peace between nations. Thus Sakharov has become a weapon of hostile propaganda against the Soviet Union and other socialist countries.

The activity of Sakharov is at its roots alien to Soviet science. It looks especially unattractive against the background of the concentration of the efforts of our whole people on the solution of the weighty problems of economic and cultural construction of the USSR, on the strengthening of peace and on the protection of the health of the international situation.

We wish to express our indignation at the statements of Academician A. D. Sakharov and resolutely condemn his activity, which is a discredit to the honour and dignity of a Soviet scientist. We hope that Academician Sakharov will think over his activities.

Signed by Academicians:

N. G. Basov	M. A. Markov	D. V. Skobel'tsyn
N. V. Bolov	A. N. Nesmeyanov	S. L. Sobolev
N. N. Bogolyubov	A. M. Obukhov	V. I. Spitsyn
A. E. Braunshtein	Yu. A. Ovchinnikov	V. D. Timakov
A. P. Vinogradov	A. I. Oparin	A. N. Tikhonov
S. V. Vonsovskii	V. E. Paton	V. M. Tuchkevich
B. M. Bul	B. N. Petrov	P. N. Fedoshev
N. P. Dubinin	P. N. Pospelov	I. M. Frank
N. M. Zhavoronkov	A. M. Prokhorov	A. N. Frumkin
B. M. Kedrov	O. A. Reutov	Yu. B. Khariton
M. V. Keldysh	A. M. Rumantsev	M. V. Khrapchenko
V. A. Kotelnikov	L. I. Sedov	P. A. Cherenkov
G. V. Kurdyumov	N. N. Semenov	V. A. Engel'gardt
A. A. Logunov		

(From *Pravda*, August 29, 1973)

HAVING familiarized ourselves with the letter of the members of the Academy of Sciences of the USSR, published in *Pravda* on August 29, we, Soviet composers and musicians, entirely agree with their estimate of the activities of A. D. Sakharov, directed against the policy of the Soviet Union on relieving international tension, and his libellous statements regarding socialist reality. It is no coincidence that the reactionary Western press rapturously seizes on these anti-Soviet "revelations".

We feel profound indignation at the actions of A. D. Sakharov, which are incompatible with the lofty title of Soviet citizen and public figure in Soviet science. His publications in the Western press are a disgrace to the honour and dignity of the Soviet intelligentsia (all the thoughts and aspirations of which are always continuously connected with the building of a new society) and the fight of the Soviet people for peace and for the extension of scientific and cultural cooperation with all countries.

Among us, representatives of Soviet art who are ever faithful to the high humanist ideals of rapprochement and mutual cooperation between nations and who unanimously support the policy of our Communist Party, the behaviour of A. D. Sakharov evokes a feeling of indignation and angry condemnation.

Signed by:

D. Kabalevskii	A. Kholminov
K. Karaev	T. Khrennikov
P. Savintsev	D. Shostakovich
G. Sviridov	R. Shchedrin
S. Tulikov	A. Eshpai
A. Khachatryan	B. Yarutovskii.

(From *Pravda*, September 3, 1973)

As *Nature* was going to press, news came of the first public defence of Sakharov by anyone associated with the Soviet Academy. Mr Igor Shafarevich, an algebraist and a corresponding member of the Academy, issued an open letter in which he praised the conscience of a man who could speak out against the "vices and sores" of Soviet society.

OLD WORLD

Unit Proposed for *in vitro* Fertilization

FRUSTRATED in their attempts to obtain financial support for their work on *in vitro* fertilization, Dr R. G. Edwards of the University of Cambridge and Mr Patrick Steptoe of Oldham General Hospital have set up a charitable trust to attempt to increase the pace of their work in helping infertile women.

The trust, entitled the Edwards-Steptoe Research Trust, will soon be launching a campaign to obtain money, and Mr Patrick Steptoe, a Consultant Gynaecologist at Oldham said this week that they would need a sum of between £100,000 and £200,000 to be viable.

The aim is to set up a unit, probably at a university, where the work on infertile women could continue at a much greater pace than now possible. Mr Steptoe and Dr Edwards now treat between 4 and 6 patients a month at Oldham but according to Mr Steptoe if he and Dr Edwards worked at the same hospital they could treat many more patients and the remaining problems could then, in all probability, be solved within a reasonable time.

The concept of a unit to carry out work on *in vitro* fertilization was suggested by Dr Edwards and Mr Steptoe a few years ago but they have had difficulties in obtaining sufficient funds for long term research. Since then, however, a great deal of work has been done on fertilization of animals *in vitro* followed by successful implantation which makes the clinical application in humans possible as well as contributing to fundamental research.

The growth of a human embryo in a test tube, which was hailed with such publicity a few years ago, was pioneered by Dr Edwards and Mr Steptoe. This work which is primarily designed to help wives who cannot have children by normal means also has several other beneficial effects not the least of which is to obtain a greater understanding of congenital abnormalities. The infertile women who can be helped by *in vitro* fertilization have at least one healthy ovary and a healthy uterus, but their Fallopian tubes which channel the ova between these organs are either diseased or non-existent. The objective of the work is to simulate, outside the body, the processes which take place in the Fallopian tubes. To achieve this ova are removed from the wife by laparoscopy—a minor operation where a needle is inserted into previously prepared ovaries through the navel in order to remove the ova. The ova are fertilized by the husband's sperm and then grow for a matter of days in the laboratory.

The process thus far has been perfected but the problems of implanting the few days old embryo in the womb to grow and develop normally are so far unsolved. One of the difficulties is that the hormone balance in a woman who is a few days into pregnancy is poorly understood and for the embryo to implant itself in the womb this hormone balance must first be controlled. And there are other problems to be solved such as the techniques of implantation, but these are of lesser importance.

Last week Professor Carl Wood of Monash University, Victoria, Australia reported that an attempt to implant a fertilized egg in the womb had failed after nine days. Mr Steptoe said this week that he and Dr Edwards had been attempting to implant embryos for over a year now without success. He said that a possible implantation for nine days could not be considered a pregnancy and that Dr Edwards and he had managed to implant an embryo for

21 days before the onset of menstruation demonstrated that the woman was not pregnant.

Mr Steptoe estimates that there are at least 20,000 women in Britain who want a child but are unable to become pregnant because of lesions affecting their Fallopian tubes. In the United States the number is probably much higher—possibly approaching a million. In Britain there are of the order of 10 million women of reproductive age, one million of whom are pregnant and one million of whom are actively avoiding pregnancy, while a further one million are trying to have a child according to Mr Steptoe.

The research trust, if enough money became available, would set up a unit where full facilities including endocrine assays and facilities for treating patients rapidly would be available. The advantages of having a unit set up in one place is that apart from being able to treat more patients than now possible a laboratory could be set up to provide on the

SOVIET OPPRESSION

IUPAP Retaliates

MORE than 200 scientists at an International Union of Pure and Applied Physics conference in Amsterdam last week signed a round-robin letter to Professor M. Keldysh, President of the USSR Academy of Sciences protesting that Professor A. V. Voronel, an expert in specific heat measurements, had been prevented from attending the conference to deliver an invited paper.

Dr Voronel was one of the seven Soviet Jews who held a hunger strike in Moscow recently in protest against the treatment of those who want to emigrate to Israel.

Professor L. Kerwin, Secretary-General of IUPAP also sent a personal telegram of protest to Professor Keldysh. Physicists at the conference were particularly annoyed by the attitude of Professor Keldysh when he was asked to help sort out the problem. Dr Voronel was invited to address the conference (the van der Waals Centennial Conference) almost a year ago. When it became plain that he was having difficulty obtaining permission to attend, Professor de Boer of the Royal Netherlands Academy of Sciences wrote to Professor Keldysh. Keldysh replied that as Voronel no longer holds a post in a Soviet institute (he resigned as

director of a division in the Institute for Physics and Radiophysics when he applied for permission to emigrate to Israel a year ago) he could not be of any help to the conference.

A fortnight ago Professor Voronel and Professor Mark Azbel were also prevented from attending an IUPAP conference in Moscow on magnetism. A number of those attending the conference retaliated by holding a session in Professor Voronel's flat, the meeting being chaired by Professor E. Stanley of MIT.

After the meeting Professors Azbel and Voronel issued a statement decrying the Soviet authorities' intervention, but also attacking the IUPAP for supporting the Soviet authorities. "We were flabbergasted" they say "to learn that (the Soviet authorities) have found unexpected support in their decision to decline our participation—support from the leaders of IUPAP." The statement is not entirely clear on what form this support has taken, but Professor L. Kerwin, IUPAP's secretary general, said this week that "this is nonsense". IUPAP has always protested against attempts to limit the free flow of information amongst scientists. IUPAP policy on this is quite clear, he said.

The union is in fact to have a meeting in Budapest later this month to discuss the problem. Israeli scientists will be consulted before it takes place.

spot hormone assays. At present in Oldham, Mr Steptoe and Dr Edward have to rely on assays produced by a commercial company, with a consequent delay of 24 to 36 hours in obtaining the results. With timing of such importance in the entire process, an immediate assay from either blood or urine would be a great boost to the work.

SELECT COMMITTEE

Critical Comment

SPARING no feelings, the Select Committee for Science and Technology has laid the blame for the demise of Tracked Hovercraft Limited (THL) on the doorstep of what seems to be everyone associated with the project. In the committee's report on THL published yesterday (*Third Report from the Select Committee on Science and Technology, Tracked Hovercraft Limited*, Commons Paper 420, £0.365), the National Research Development Corporation, the Department of the Environment, and the Department of Trade and Industry, as well as various managers and ministers, emerge with far from unblemished reputations. But Professor Eric Laithwaite of Imperial College emerges as the poor, downtrodden hero of the entire affair.

The National Research Development Corporation is criticized for being a poor employer—"in our view the conduct of the NRDC towards the staff of THL falls far short of that expected of a good employer", and for not taking "sufficient action to obtain a definite decision from the government during 1971 and 1972". The select committee is also convinced that the structure of the NRDC is in need of "urgent financial review". In the light of the THL experience the select committee feels that the financial constraints placed on the corporation are excessive and that "the existing organization is ill-suited to the management of complex developments". Accordingly, the committee feels that the time is ripe for a high level review of the "organization needed to develop innovation" and the terms of reference of the review should be wide enough for the organization of government departments to be considered also.

The Department of Trade and Industry is criticised for its procrastination during the entire affair, and the committee admits that it was startled by the department's action "in making over to a private company [Hawker Siddeley] on a preferential basis, technology either owned by NRDC or existing at Imperial College".

The select committee states that in its opinion the "Minister for Transport Industries and his Department evidently had no interest in the THL company whatever and precious little in the technology". "It is incomprehensible", continues the committee, "that the

department responsible for future transport requirements remained so aloof from an advanced transport project".

But Professor Eric Laithwaite, who held the committee spellbound when he gave evidence, is compared with Sir Frank Whittle. The attitude of official witnesses to Professor Laithwaite, says the committee "was an uncomfortable reminder of the treatment of Sir Frank Whittle by earlier governments". It is of little surprise that the committee recommends that the government should accept the proposals made by Professor Laithwaite and Imperial College that a "multi-user test facility" be set up at Earith under the auspices of Imperial College "for the testing and development of large linear motors, high speed suspension systems and related technology". But the committee are only too aware that this suggestion was given a most cool reception by Mr Michael Heseltine, Minister for Aerospace, when he appeared before them in July.

Mr Heseltine had his knuckles rapped by the committee for his handling of the announcement of the closure of THL. On February 12, Mr Heseltine announced in the House of Commons that the government was still considering whether or not to provide financial assistance to continue work at Earith, but evidence obtained from Mr Heseltine two days later revealed that the decision to cease work at Earith had

been taken on January 29. The committee concludes that "Mr Heseltine's answer to Mr Stoddart's question . . . was therefore untrue".

But THL is now defunct and nothing that the select committee can do can resurrect the project in its original form. The committee is very concerned that such an occurrence should not happen in future and it again raises one of its favourite hobby horses that there should be a minister in the cabinet who "would bring about a significant improvement in the management of research and development".

The track still exists at Earith and the select committee feels that it should be put to use immediately. The government, says the committee, should defray any costs connected with the retention of the site and its equipment and the completion of the test track. The government should also meet the initial running costs of the centre by way of direct annual grant, including the costs of a permanent staff which will be responsible for the day to day management of the site.

The committee, as a nudge to Dr Dahrendorf, who has recently produced a policy for research in Europe based on collaborative research programmes, recommends to the government that it should use the facilities at Earith "within a European Research and Development programme".

ARBORICULTURE

1974—Save a Tree Year?

PRESSURE is mounting for an increase in research on amenity trees. Following the recent ravages of Dutch Elm disease in Britain, an independent working party on research priorities for amenity trees was set up by the Association of British Tree Surgeons and Arborists last year. It is due to report shortly.

The working party—which includes members of the Forestry Commission, the Natural Environment Research Council, the Association of British Tree Surgeons and various other interested bodies—has taken as its remit a complete review of the situation in Britain, which, is so impoverished that only about £10,000 a year is spent on research on amenity trees other than elms, which have received more attention since the Dutch Elm epidemic.

But Dutch elm disease is only part of the story. Dr R. G. Pawsey of the Department of Forestry, University of Oxford and a member of the working party, pointed out in a lecture to the British Association annual meeting recently, that other diseases—beech bark disease, sooty bark disease among sycamores, ash decline and honey

fungus—are wreaking great damage among amenity trees. And this in a year in which the British public is being exhorted to plant trees.

Estimates of the damage caused by these diseases is largely guesswork as no survey of them has ever been undertaken. The fear is that too little will be done too late, for lack of basic information about amenity trees. Professor F. T. Last, Director of the Natural Environment Research Council's Institute of Tree Biology and chairman of the working party, is a known advocate of greater expenditure on amenity tree research, and it seems likely that when the working party's report emerges later this year or early next, it is going to demand that more money be spent on an area of research that has been largely ignored in Britain. The working party has not reached any conclusions yet, but it is known that there is a high measure of agreement between the members of the committee as to what should be done.

The need is clearly felt among those in the field for a research unit specifically to study amenity trees, for a regular survey of disease among these trees, and for an information service to tell local authorities and the public what to do about amenity tree diseases.

NEW WORLD

Oceanography up the Creek

by our Washington Correspondent

In language remarkable for its bluntness, a Presidential advisory committee has castigated the federal government's policies for marine research and ocean resource management. The criticism, which centres chiefly on the fact that too many separate agencies have their fingers in the marine pie, is contained in a report made public last week by the National Advisory Committee on Oceans and Atmosphere (NACOA), a 25-member committee which met under the chairmanship of Dr William Nierenberg, Director of the Scripps Institution of Oceanography. The committee also expressed its alarm at recent cutbacks in funding for oceanographic research.

Three years ago, the National Oceanic and Atmospheric Administration (NOAA) was set up with high hopes that it would consolidate and coordinate the federal government's activities in marine and atmospheric affairs. But from the start it was clear that NOAA would be incapable of bringing complete order out of the chaos because many important programmes and activities remained out of its control—off-shore oil, gas and mineral resource exploitation and management were left in the hands of the Department of Interior, for example, and responsibility for marine and atmospheric sciences is now spread over some nine departments and agencies. In short, NACOA suggests that there are "too many actors, too many separate chains of command, too many crosscutting policies, too many separate budgets, appropriations and programs. In this confusion, national priorities have no perspective and neither the Executive Branch nor the Congress is in a position to lead effectively, much less enforce accountability for results".

There have, however, been a host of coordinating committees set up in the past few years to help overcome the fragmentation. But "coordination is never enough", NACOA suggests, for "rarely does it involve table-pounding establishment of priorities, guidelines and new policies to meet new problems. Especially when the budget gets tight, coordination is not by itself tough enough to protect multiagency programs". The truth of that statement can be seen from the impact of budgetary cutbacks on oceanography.

On Christmas Day last year, the crew of the *Eltanin*, a research ship operated by the National Science Foundation, received word that their vessel would be

mothballed at the end of the voyage. Along with two NOAA oceanographic ships, the *Surveyor* and the *Discoverer*, which were laid up earlier this year, the *Eltanin* has been taken out of service because of cutbacks in federal spending imposed by President Nixon as part of his strategy for holding down taxes. The decimation of the oceanographic research fleet which NACOA reckons amounts to a reduction of about 25 per cent—has come, the committee says, "at a time when a long-cultivated collaboration between oceanographers and meteorologists is just beginning to show results and joint programs with foreign scientists are just beginning to materialize". The reduction in the fleet, NACOA believes, "will have pervasive and long-term effects".

Another example of potentially damaging cutbacks in marine activities is the fact that the Coast Guard has been forced to abandon three ocean data gathering stations this year, and will have to abandon three more next year. The agency will then be left with a single station, manned for only 8 months a year. Yet, as NACOA points out, the stations' functions of offshore weather and ocean monitoring are

"becoming more rather than less important to seagoing activities". To be sure, the functions of the Coast Guard stations are being taken over to some extent by satellite surveillance and by NOAA's data buoy programme, but NACOA suggests that these programmes will not be ready in time to fill the gap. In other words, "what was a least harm cut to the Coast Guard was a far more serious one to an interagency program in the oceans and atmosphere which, in a certain sense, belonged to no one".

The Administration's response to these charges, in the form of a set of comments prepared by Frederick B. Dent, Secretary of Commerce, is that although the cutbacks are painful, no programme of "overriding national importance" has been sacrificed. Nevertheless, Dent announced that the concerns of NACOA "will be reviewed to see whether some restoration should be made in fiscal year 1975 and beyond". As for the oceanographic fleet in particular, Dent said that the cutback amounts to only 18 per cent, rather than 25, but he has in any case asked the chairman of the Federal Council for Science and Technology (Dr Guyford

WEATHER MODIFICATION

Advice Ignored

by our Washington Correspondent

A YEAR ago, the National Advisory Committee on Oceans and Atmosphere made known its concern about the shortage of funding and lack of direction in the federal government's weather modification programmes. The committee recommended, among other things, that more attention should be paid to basic research, that the National Oceanic and Atmospheric Administration should be given more of a leading role in developing policies and strategies for weather modification, and that Project Stormfury, a hurricane modification project, might be better moved from the Atlantic to the Pacific. The committee said in another report, published last week (see above), however, that few of its recommendations have been taken up, and in fact, some steps have been taken in the opposite direction.

For one thing, the Bureau of Reclamation, in the Department of Interior, has been given chief responsibility for the federal government's programmes in precipitation enhancement, and the budget has been cut in half. "It is important to note", NACOA said, "that

precipitation enhancement is not ready for general operational use, and will not be, without much greater effort in research". Moreover, the funding for basic and field work in atmosphere and cloud physics has declined "to the point where the small cadre of experts built up over the last twenty years is in danger of being dispersed". Project Stormfury has also been suspended because of budgetary cutbacks, and will not be reinstated until sufficient numbers of suitably equipped aircraft are made ready.

Frederick B. Dent, Secretary of Commerce, has replied to NACOA's complaints by saying that although they are "appreciated", some of them have already been taken care of. He pointed out, for example, that there have been substantial increases in some areas of funding for weather modification, particularly for purchasing capital equipment such as heavy aircraft. Dent did not, however, address himself to NACOA's complaints about lack of direction in the programme, or to the more fundamental charge that "there is a danger that the funding authorities, in their quite proper zeal for practical results, will underestimate and undervalue the still extensive research that must precede reliable operational use".

Steuer, Director of the National Science Foundation, which has a significant stake in the enterprise) to make a study of the effects.

NACOA also believes that the fragmentation in the federal government's management of marine affairs has important implications for resource management and exploitation. In particular, it is not helping development of a strategy for exploiting oil and gas deposits on the outer continental shelf, and there is confusion over who has the chief responsibility for controlling the development of deep water ports.

But the committee does see a way out of the tangled situation in marine affairs—creation of a cabinet-level Department of Natural Resources which would bring together all the programmes that have a bearing on ocean and atmospheric affairs and place them in a single division. President Nixon has proposed the setting up of such a department—the Department of Energy and Natural Resources—but the fate of the proposal, which is now being considered in Congress, is anybody's guess. With the help of the growing concern over the energy crisis, however, it is generally considered that legislation setting up an umbrella department will emerge from Congress in some shape or form next year.

OIL SHALE

Rootin' in the Rockies

by our Washington Correspondent

In the next decade or so, a remote area of the Rocky Mountains is likely to undergo a dramatic transformation. At present a ranching and hunting area, it will probably become more and more industrialized and its population could almost double by 1985. The reason is that it is sitting on perhaps the richest oil shale field in the world, and the rapidly escalating cost of petroleum is making its exploitation increasingly attractive to the oil industry. Surprisingly, however, proposals to open up the oil shale fields to commercial development have so far sparked little opposition from environmentalists, and they have certainly failed to achieve the notoriety of ventures such as the trans-Alaska pipeline.

The shale is concentrated in a formation centred in the region where Colorado, Utah and Wyoming meet, and it is reckoned to contain some 1,800 (US) billion barrels of crude oil. Of that total, the National Petroleum Council reckons that about 130 (US) billion barrels are contained in deposits that can now be exploited economically, while the Department of Interior puts the figure at about 600 (US) billion. By contrast, proven reserves of oil from other sources in the United States are

reckoned to be about 425 (US) billion barrels.

Oil shale consists of organic deposits called kerogen, locked into rock. To extract oil from the shale requires a process involving mining the shale, crushing it up and heating it in a closed retort; since it is such a costly process compared with drilling for conventional petroleum, the price of petroleum has to be right before exploitation becomes economically attractive. Nevertheless, it is widely expected that sizable oil shale exploitation will take place over the next decade or so, and the Department of Interior is hoping to lease six tracts of land—two in each of Colorado, Utah and Wyoming—for prototype commercial production. Last week, the department published its final analysis of the likely environmental effects of the prototype programme, and it also took a speculative look at the environmental damage likely to result from possible full-scale exploitation of the field.

A massive, six volume set of documents running to some 3,200 pages, the environmental impact statement clearly shows that even the prototype programme will significantly alter the characteristics of the area, and that full commercial production will essentially change it from a rural to a semi-industrial region. The prototype programme is designed to allow the oil industry to develop the technology for full commercial exploitation of the shale field, and it will probably result in production of up to 250,000 barrels of shale oil a day. The department reckons, however, that as much as 1 million barrels of shale oil a day could be produced from the region by 1985 if the prototype programme proves the feasibility and acceptability of commercial production.

As for environmental damage, the environmental impact statement shows that although the mining and processing operations will have to conform to present federal and state environmental laws, there will be irreversible effects. The mining operations themselves will cause some damage, particularly in one tract in Colorado which will be surface mined—the others will be underground operations—but disposal of the crushed rock after removal of the shale oil will present a more difficult problem. Some of the rock will be replaced in the mine shafts, but the excess will be used as landfill. There will also be adverse effects on air quality, resulting from dust and, more important, from sulphur dioxide emissions from the retorts.

But one of the chief problems is likely to arise from the use of water in the extraction and processing operations. The region is arid, and already water is not in particularly plentiful supply. Thus, the impact statement suggests that ultimately the availability of water could limit commercial exploit-

ation to about 1 million barrels of shale oil a day.

In the long run, however, the chief irreversible effect on the environment is likely to stem from the fact that oil shale production will attract many people to the area, resulting in a population boom which will result in nearly 115,000 people living in the region by 1985, assuming full-scale commercial production is under way by then. In 1970, the seven counties that comprise the oil shale region had a total population of only 119,000. As the impact statement points out, "that oil shale basins are characterized by their wildlife resources, essentially undeveloped natural resource condition, and limited human activity. . . . Thus the remote and primitive character of parts of (the region) will be lost".

TENURE

Getting Locked In

TENURE has been coming under increasing fire in the United States in the past few years, particularly in colleges and universities faced with rapidly escalating costs and a preponderance of red ink on the balance sheet. Administrators in such institutions have complained that the growing proportion of teaching staff with tenure—appointments for life—is gradually locking higher education into a set structure because it is making it difficult for institutions to respond to changing enrolment patterns by phasing out some courses and introducing or expanding others. Their complaints have now been put into perspective by a survey carried out by the American Council on Education*. According to responses from more than 42,000 college and university teachers, the percentage of faculty members holding tenured positions has increased from 46.7 to 64.7% in the past four years. One result of the trend is that some institutions have been forced to dismiss faculty members rather than give them tenure.

Alongside the increase in tenured faculty, the survey has brought to light the fact that the average age of academic teaching staff has increased (58.7% are over the age of 40, compared with 54.4% four years ago) and the proportion of faculty members holding associate or full professorships has also gone up (from 42.3 to 50.7%). Women and minority groups have also increased their representation on university and college teaching staffs, but hardly by leaps and bounds—the survey shows that their numbers grew from 19.1 to 20%, and from 2.2 to 2.9%, respectively.

**Teaching Faculty in Academe: 1972-73*, \$3.00. Available from ACE, 1 Dupont Circle, Washington DC 20036.

NEWS AND VIEWS

Hydromechanics of Fish Migration

IN a note in this issue of *Nature* (page 48) Weihs discusses some aspects of the energetics of fish locomotion and concludes, on theoretical grounds, that it would be advantageous to swimming fish, in terms of energy of movement as a proportion of a given total energy store, if their speed through the water was maintained at about one fish length per second. Independent experimental evidence, not referred to by Weihs, suggests that this swimming speed may indeed have some physiological significance: for it is at prolonged swimming speeds, above that of one fish length per second—where it has been shown in the coalfish *Gadus virens*—that the diameter of the white (anaerobic) muscle fibres starts to increase.

Studies on schooling or shoaling of fishes have suggested that there is an upper limit to the searching speed of predators and that they could achieve this digit at speeds less than the cruising speed of three lengths per second established by experimental observations. The underwater films of tuna from the RV Townsend Cronwell give the impression of rather slow and idle swimming between meals.

Weihs's conclusions suggest that the extent of a migratory circuit could depend on the size of the fish. Furthermore, as the migratory movements themselves—for example, feeding area to wintering area, and wintering area to spawning area—are seasonal, the distances between the area could be similarly limited. The figures in the table below show the distances that various fish could cover each day when swimming at 1 length s^{-1} .

It is known that herring in the North Sea may migrate about 600–800 miles yr^{-1} on a straight course and that the Arcto-Norwegian cod may cover 1,500 miles yr^{-1} . Hence the least period spent on migration is 50–70 days for the herring and 30–60 days for the cod (or one sixth of the year). A straight course migration implies some orientation to a current, which is unlikely. On more devious courses the fishes may spend a much greater part of their lives in migration. The migratory range could perhaps be extended by behavioural or physiological adaptations; for example, the distance travelled could be substantially increased if the direction of migration was downstream in the direction of the main ocean currents, rather than against them. And in areas where tidal currents are significant, economies could be achieved, and the range of migration extended, if the fish joined those tides which would carry it in the direction of migration.

Length (cm)	Species	Speed in knots	Distance covered in 24 h (nautical miles)
10	Sprat	0.2	4.8
25	Herring	0.5	12.0
50	Small cod	1.0	24.0
100	Big cod	2.0	48.0
150	Tuna	3.0	72.0

A comparison between a cod and a salmon suggests a line of physiological adaptation. Both fish, when sexually mature, are of similar size, say 70 cm. Both species undertake long spawning migrations; mature Arcto-Norwegian cod move from Bear Island to the west Fjord, from 74° N

to 68° N, and those salmon which spawn in the headwaters of the Fraser River, British Columbia, move from 49° N to 55° N, and then after having returned to their coastal waters from the high seas. But although cod in the sea might have to swim against a 1 knot (50 cm^{-1}) current, the salmon in freshwater must swim against a very much faster flow and one suspects that a cod-like fish would not undertake the migration carried out by a salmon of similar size unless it was designed somewhat differently. It is well known that the muscle systems of the two species are very different.

The energy devoted to swimming is equal to that devoted to maintenance. Growth is proportional to converted food less maintenance. Hence it might be expected that the more migratory fish grow less than the less migratory ones. Plaice migrate about half the distance of herring and are much larger. But such comparisons cannot be extended very far.

Weihs's argument draws attention to the fact that very little is known about the locomotory behaviour or swimming performance of fish on migration: the information from tagging returns is very limited. Recent advances in sonar techniques, both in North America and the United Kingdom, however, provide the means of tracking individual fish in lakes, rivers and the open sea for periods up to several days. Data collected from such studies should go some way to fill in some of the gaps in knowledge.

From a Correspondent

Magnetic Fields Adrift

THAT versatile genius, Edmond Halley, astronomer, geophysicist, navigator, and surveyor, made the first extensive geomagnetic survey and drew up the first chart of magnetic declination, covering much of the Atlantic Ocean. He noticed that the secular variation of the field, which had already been detected for London by Gellibrand, a Gresham Professor of Astronomy, behaved as if the Earth's field were rotating in a westerly direction relative to the surface of the Earth. Halley then went on to propose a model of the Earth which is in some ways remarkably like the model now accepted of a core surrounded by a mantle.

There the matter rested, attracting little attention until, some two and a half centuries later, Sir Edward Bullard re-examined the secular variation in Africa. He and his colleagues showed that the secular variation could be accounted for by electrical currents flowing in closed loops near the surface of the liquid, electrically-conducting core of the Earth, the whole system drifting westwards relative to the surface of the Earth. Apart from its account of the secular variation, this conclusion proved to be of great importance because it led Bullard himself, as well as others, to examine the possibility that the main field of the Earth might be generated by electrical currents maintained in the core by some sort of dynamo action.

Malin and Saunders (see page 25 of this issue) have now re-examined the secular variation to see whether, as

hitherto assumed, the field appears to rotate about the geographical axis of the Earth, or whether a rotation about some other axis best represents the observed changes. They find that indeed the apparent rotation is about an axis which differs somewhat from the geographical axis, and not only that, but that the direction of the axis about which the field appears to rotate has undergone a steady change from near Novaya Zemlya in 1945 to near the geomagnetic pole north of Canada in 1965. Malin and Saunders maintain that the departures from the geographic axis are significant, and they go on to develop the implications of their results for the dynamics of the Earth. They argue that the difference they find implies that the angular momentum vectors of the core and mantle point in slightly different directions, rotating about the direction of the total angular momentum which is of course fixed in space.

Thus, it should be possible to observe a slight displacement of the pole of rotation of the mantle (that is, of observatories on the surface) from a direction fixed in space. This conclusion is of particular interest just now, for although the effect is at the limit of detection by conventional astronomy, it amounts to a daily variation of apparent position with an amplitude of some 5 cm, and may therefore be detected by laser ranging to the Moon.

Most aspects of the rotation of the Earth and the variations it undergoes are as obscure now as when Munk and MacDonald wrote their stimulating monograph (*The Rotation of the Earth*; Cambridge University Press, 1960). The work of Malin and Saunders calls attention to two means of studying variations of the rotation (the analysis of the geomagnetic field and laser ranging to the Moon), and may thereby help to elucidate the forces, whether of internal or external origin, which drive those variations.

From a Correspondent

Maternal Messengers

A MINOR revolution in molecular biology which has accompanied two recent discoveries is nowhere more apparent than in the study of messenger RNA and protein synthesis in early embryonic development. First, the discovery that most messenger RNA molecules contain a poly (A) fragment has led to the development of methods for their purification by affinity chromatography beyond the dreams of even the most optimistic people a few years ago. Couple this with the discovery of reverse transcriptase, an enzyme which will copy RNA sequences primed by oligo (A,T) faithfully into DNA sequences which can then be used in DNA-DNA hybridization reactions and so allow a simpler interpretation of the data. Add to these such useful things as the modern, low background cell-free protein synthesizing systems and one has an almost complete tool kit for investigating one of the central problems in developmental biology today, namely the question whether or not the embryo inherits a maternal store of messenger RNA for use in protein synthesis during early development. If so, how is it used?

Experiments on sea urchin and frog eggs and embryos during the past ten years led to the formulation of the so-called maternal messenger hypothesis. It was shown that in sea urchin embryos after fertilization there is an increase in the rate of protein synthesis (Epel, *Proc. natn. Acad. Sci., U.S.A.*, **57**, 899 ; 1967) which is accompanied by the

conversion of monoribosomes to polyribosomes (Rinaldi and Monroy, *Devl Biol.*, **19**, 73 ; 1969) and that until the late blastula stage this increase is not dependent on the presence of a nucleus, which could either be physically removed (Denny and Tyler, *Biochem. biophys. Res. Commun.*, **14**, 245 ; 1964) or chemically inactivated by actinomycin D (Gross, Malkin, and Moyer, *Proc. natn. Acad. Sci., U.S.A.*, **51**, 407 ; 1964) without impairing protein synthesis. Similar observations have been made for other invertebrates (Bell and Reeder, *Biochim. biophys. Acta*, **142**, 500 ; 1967 ; Gould, *Devl Biol.*, **19**, 482 ; 1969) with slight differences in regard to the timing and magnitude of the effects.

A careful study of protein synthesis in *Rana pipiens* eggs and embryos (Smith, Ecker and Subtelny, *Proc. natn. Acad. Sci., U.S.A.*, **56**, 1724 ; 1966) showed that enhanced protein synthesis can be detected in eggs during maturation before fertilization is possible but that fertilization itself does not result in any major quantitative change in the rate of protein synthesis. It was also shown that the presence of a nucleus is not required for the enhanced synthesis during egg maturation (Ecker and Smith, *Science, N.Y.*, **160**, 1115 ; 1968) nor for its continuation after fertilization (Smith and Ecker, *ibid.*, **150**, 777 ; 1965). It would seem that amphibians differ from invertebrates in that the increase of protein synthesis occurs before fertilization but that the two groups are similar at these early stages of embryogenesis in the lack of a requirement for direct genomic control over protein synthesis.

The discovery of mitochondrial DNA in eggs and embryos (Dawid, *Proc. natn. Acad. Sci., U.S.A.*, **56**, 269 ; 1966 ; Piko *et al.*, *ibid.*, **59**, 838 ; 1968) and the demonstration of cytoplasmic RNA synthesis resistant to actinomycin D in sea urchin embryos (Selvig *et al.*, *Devl Biol.*, **22**, 343 ; 1970 ; Craig, *J. molec. Biol.*, **47**, 615 ; 1970) led to doubts concerning the validity of the interpretation of results on protein synthesis in enucleate and actinomycin D treated embryos. Work by Craig and Piatigorsky (*Devl Biol.*, **24**, 214 ; 1971) showed, however, that even in enucleate sea urchin embryos treated with ethidium bromide, in which cytoplasmic RNA synthesis is severely curtailed, protein synthesis at fertilization is only slightly affected and the nature of the proteins made is not changed at all. Although ethidium bromide causes morphological abnormality in these embryos the general conclusion that neither nuclear nor mitochondrial RNA synthesis is required for the increased translation at fertilization seems valid. Chase and Dawid (*Devl Biol.*, **27**, 504 ; 1972) found that in *Xenopus* embryos mitochondrial RNA synthesis starts at gastrulation and therefore can have no influence on protein synthesis in early development.

Further evidence in support of the maternal messenger hypothesis comes from RNA/DNA hybridization experiments. Davidson and Hough (*J. molec. Biol.*, **56**, 491 ; 1971) showed that approximately 0.1% of the RNA in a mature *Xenopus* oocyte hybridizes to 1.2% of the unique fraction of the *Xenopus* genome. If hybridization to unique DNA is an adequate criterion for identifying messenger RNA then the mature *Xenopus* egg contains enough mRNA to code for 30,000 different proteins with messages of the size of globin mRNA (molecular weight 200,000).

Recently Slater, Slater and Gillespie (*Nature*, **240**, 333 ; 1972 ; *Proc. natn. Acad. Sci., U.S.A.*, **70**, 406 ; 1973), using hybridization of poly (U) to detect poly (A) sequences, have demonstrated the presence in unfertilized sea

urchin eggs of an RNA fraction with a heterogeneous sedimentation profile containing poly (A). A similar RNA fraction is also present in fertilized eggs. It seems that the size of the poly (A) fragment in unfertilized eggs is one half of that found in two cell embryos and the doubling in size occurs at the end of the one cell stage just before the first cleavage. This doubling is not correlated with the increase in protein synthesis which takes place soon after fertilization and is not dependent on continued protein, nuclear or cytoplasmic RNA synthesis. The poly (A)-containing RNA has been localized in the unfertilized egg in a poorly characterized subribosomal fraction which rapidly shifts in fertilized eggs into a so-called ribosomal fraction.

Slater *et al.* were able to show that exogenous poly (A) does not bind fortuitously to RNA nor to ribosomes from newly fertilized eggs. Although the poly (A)-containing RNA and ribonucleoprotein particles described in these two articles are inadequately characterized, the work does suggest that unfertilized sea urchin eggs contain a population of mRNA molecules which shift from a subribosomal fraction, presumably inactive in protein synthesis, into a ribosomal fraction, presumably active in protein synthesis, in newly fertilized eggs. Epel (*loc. cit.*) observed in the sea urchin *Lytechinus pictus* (the animal used by Slater *et al.*) that after fertilization there is a lag of 5–6 min before a detectable increase in incorporation of ¹⁴C-leucine occurs. It is clearly important to know how well correlated the increase in protein synthesis is with the shift of poly (A) RNA to the ribosomes.

Epel's paper also suggests that the increase in relative rate of protein synthesis at fertilization is five times, but Slater *et al.* show that 30 min after fertilization the embryo contains only 2.7 times more poly (A) RNA associated with ribosomes than the unfertilized egg. If these estimates are a fair measure of mRNA active in translation at these two stages it must be concluded that the mRNA of unfertilized eggs is translated less efficiently than that of fertilized eggs.

This conclusion does not agree with observations by Humphreys (*Devl Biol.*, **20**, 435; 1969) who, also using *Lytechinus pictus*, investigated the possibility that fertilization results in increased utilization of mRNA, based on higher rates of initiation or elongation of nascent chains, rather than increased availability. One consequence of an increased rate of initiation would be an increase in the average size of the polyribosomes and he was able to show that this does not occur—polysomes are the same size before and after fertilization. A consequence of an increased rate of elongation would be decreased transit times from initiation to termination provided the size of the mRNA is the same. He was able to show that the average size of the proteins does not change and therefore presumably neither does the average size of the messages being translated and, moreover, the average time for the synthesis of a protein is the same in both unfertilized and fertilized eggs. He also estimated that the increase in relative rates of protein synthesis was fourteen times, a value almost three times greater than Epel's estimate, and concluded that the only explanation for his results can be that fourteen times more mRNA is used after fertilization than before. This suggests that the mechanism which controls increased protein synthesis at fertilization is not based on some lesion in the protein synthetic machinery itself but on the increased availability of a set of mRNA molecules.

Investigation of the nature of the mechanism controlling the onset of increased protein synthesis at fertilization in sea urchins has chiefly centred on examining the different components of the apparatus for protein synthesis for defects and inhibitors. Suggestions range from inadequate activation and availability of transfer RNA (Maggio and Catalano, *Archs. Biochem. Biophys.*, **103**, 164; 1963; Glisin and Glisin, *Proc. natn. Acad. Sci., U.S.A.*, **52**, 1548; 1964; Ceccarini *et al.*, *ibid.*, **58**, 2235; 1967) through inhibited or inefficient ribosomes (Hultin, *Expl Cell Res.*, **3**, 494; 1961; Candelas and Iverson, *Biochem. biophys. Res. Commun.*, **24**, 867; 1966) and inactive polysomes or mRNA ribosome complexes (Piatigorsky, *Biochim. biophys. Acta*, **166**, 142; 1968; Monroy *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **54**, 107; 1965) to inactive mRNA protein particles (Mano and Nagano, *Biochem. biophys. Res. Commun.*, **25**, 210; 1966; Spirin, *Curr. Top. Dev. Biol.*, **1**, 1; 1966). The work on tRNA availability and activation seems to be in agreement that it is not limiting protein synthesis in the unfertilized egg. Experiments to test the activity of unfertilized and fertilized sea urchin egg ribosomes *in vitro* show that there is no difference when primed with synthetic polynucleotides (Stavy and Gross, *Biochim. biophys. Acta*, **182**, 193 and 203; 1969).

Such experiments suffer from the inability to test for defects in the normal process of initiation which in eukaryotes relies on the binding of met-tRNA to small ribosome subunits before mRNA binding (Darnbrough *et al.*, *J. molec. Biol.*, **76**, 379; 1973). Recent work (Metafora *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **68**, 600; 1971; Gambino *et al.*, *Biochim. biophys. Acta*, **312**, 377; 1973) has identified an inhibitor of protein synthesis in salt washes of ribosomes of unfertilized but not of fertilized sea urchin eggs. The inhibitor reduces the rate of elongation measured in the rabbit reticulocyte cell-free system and its removal from ribosomes at fertilization is supposed to account for the increased translation observed. It should be pointed out that Humphreys (*loc. cit.*) found no difference in the rate of elongation before and after fertilization and that the stoichiometry of the inhibitor action leaves much to be desired because to obtain a 50% inhibition of poly (U)-primed phenylalanine incorporation by a given amount of ribosomes from sea urchin embryos, salt wash protein from approximately fifty times the amount of unfertilized egg ribosomes has to be added.

The best explanation for increased protein synthesis after fertilization in sea urchins lies with the maternal messenger hypothesis. At fertilization some ill-defined parameter increases the availability for translation of a store of mRNA molecules laid down in the egg during oogenesis. It seems likely that within the near future isolation of this maternal store of message on poly (U) or oligo (dT) columns will allow direct tests for its ability to promote protein synthesis *in vitro* for the spectrum of proteins for which it codes, and for its ability to hybridize with DNA. Using these direct assays it will be possible to find out if the developing oocyte synthesizes two kinds of mRNA—one which it stores and the other which it translates—or whether protein synthesis in unfertilized eggs is on a small random sample of all messages. It will be possible to tell when during oogenesis and development these messages are transcribed and translated and eventually it may even be possible to suggest in detail how the increase in protein synthesis at fertilization occurs.

P. J. F.

ANZAAS

Pepping Up the Cow

from a Correspondent

At one of the more significant intersection symposia at the ANZAAS Congress, which was held in Perth from August 13 to 17 (see also *Nature*, **244**, 534; 1973), the role of polyunsaturated fats in animal production and human nutrition was discussed. Local research in this field was given strong impetus by the development in 1967 by Dr K. Ferguson (CSIRO Division of Animal Physiology) of a method for protecting the essential amino acid methionine with formaldehyde in order to bypass the rumen of the sheep, thereby increasing the wool yield. This idea was later extended by Dr T. Scott of the same laboratory to protecting polyunsaturated fats in order to increase animal production generally.

At the ANZAAS Congress Dr Scott reported how this idea has now been developed to the point where the research and development activity for commercial application has become the largest such effort between government science and industry in Australian history. After world-wide competition, CSIRO granted licences on a world basis to a new combine, Dalgety-Agrilines Ltd, for a record fee in return for exclusive royalties. The process has been patented in many countries, and the Australians claim that there is no competition for its unique features.

The process depends on the microencapsulation of globules of lipids, 1 μ m in diameter, from sunflower or safflower oil seeds, with monomolecular layers of protein. In sheep and cattle, the protein envelope remains stable in the rumen where the pH is approximately 6, but dissolves in the abomasum (the third stomach) where the pH is 2. In the abomasum, as the protein envelope comes off, the fat is absorbed by the duodenal mucosa and is carried away by the blood stream and lymphatic system to be metabolized normally. By this device, the rumen is bypassed; the hydrogenation process which usually converts the polyunsaturated portion of the fats in pasture grass in the animal's normal food into the saturated form is thus avoided (roughly 5% of pasture grass is fat, of which about 75% is polyunsaturated).

This process makes available to the animal up to 40% of the energy obtainable from the fats, which is otherwise obtained by the bacteria in the rumen: experiments have demonstrated increases in yields of milk, butterfat content in milk and growth rates of both cattle and sheep. Moreover, by using polyunsaturated fats, as in sunflower seeds, the milk and meat products from the animal show increases in the ratio of

polyunsaturated to saturated fats. In meat, for example, there is a relative increase in linoleic acid, the polyunsaturated fat believed to have an important effect on serum cholesterol levels in the human blood. At ANZAAS, figures were quoted which showed that, after supplementing the normal food of cattle with 3 kg of protected lipid per day, for 40 days, the proportion of polyunsaturated fats in tissue fat increased from about 4% to 30%. With dairy cattle a similar effect on milk is noticeable within 3 days. Dr J. Wilson, the Technical Manager for the Australian branch of the international company which has a 3-year programme for testing and developing the process in Australia, New Zealand, the United Kingdom, the United States and Canada, told the congress that many scientific problems remain to be solved. The milk products, for example, are too easily oxidized, and optimum conditions have to be found for dairy processes such as pasteurization, curing and culturing.

The process of microencapsulation is simple and cheap to establish. A plant producing about 1 tonne per hour will cost about \$A120,000. The whole oil seed is first crushed with water and 5% casein or other protein source in alkaline solution, and the mixture is homogenized in a colloid mill causing dispersion of the fat globules in the aqueous phase. The third stage involves treatment with a small amount of formalde-

hyde which causes the protein matrix surrounding each fat globule to shrink and harden. Finally, the material is flash dried.

Conflicting evidence was presented to the congress about the medical effects of polyunsaturated foods. Dr P. Nestel (Clinical Science Department, Australian National University) reported evidence that lowering of serum cholesterol reduces the risks of arteriosclerotic heart disease. Headlines in the popular press were, however, generated by a pathologist at the same university, Dr C. West, who questioned the safety of polyunsaturated foods; he claimed that tests in the United States had shown that a person on such a diet suffers increased risk of gallstones and cancer. This claim was contested by Drs Wilson and Nestel who said that it was not new, was not based on sound evidence, and referred to the benzpyrenes and polycyclic hydrocarbons found in vegetable oils and not to polyunsaturated fats such as the ones described here.

New Copper Process

Another promising industrial process was reported to the congress by Dr A. J. Parker (Australian National University) who has recently been appointed Foundation Professor of Chemistry at the new Murdoch University in Western Australia from 1974. In his Liversidge Lecture (in memory of a pioneer Australian chemist), Dr Parker described how

Polymerases Galore

NATURE, both in reality and its journalistic reflexions, of late, it seems, abounds in DNA polymerases. The role of DNA polymerase I is the topic of a communication by Goebel and Schrempf in *Nature New Biology* next Wednesday (September 12); in the issue for August 22 Masker and Hanawalt (*Nature New Biology*, **244**, 242; 1973) discussed the excision repair function of DNA polymerase II and Youngs and Smith (*ibid.*, 240) a similar function of DNA polymerase III.

Goebel and Schrempf exploited a mutant of *Escherichia coli* which contains a temperature sensitive DNA polymerase I. Their technique was to examine the sensitivity of supercoiled DNA of colicinogenic factor E implanted in the mutant to RNA-degrading conditions (ribonuclease and alkali) after pulse-labelling and chasing with tritiated thymidine for varying lengths of time at a permissive temperature (30° C) and at a temperature (43° C) that inactivates the DNA polymerase I both *in vivo* and *in vitro*. The supercoiled DNA of the mutant, pulse-labelled at either temperature, is partially sensitive (converted to the "relaxed"

or open circular form) to RNA-degrading conditions. After chase, however, a smaller proportion of the DNA purified from the sample incubated at the lower temperature is affected by such treatment. Supercoiled DNA in the parent strain grown at either 30° or 43° C is almost completely resistant to RNA-degrading conditions. Only the small fraction of RNA-containing nascent supercoiled DNA from the parent strain is sensitive, as detected by pulse-labelling the RNA.

Goebel and Schrempf suggest that DNA polymerase I is responsible for excision of RNA involved briefly in the origin of DNA replication and subsequently replaced by DNA (see *Nature*, **244**, 483; 1973). Their interpretation is consistent with the known exonucleolytic activities of DNA polymerase I. Once the RNA is excised DNA polymerase I also apparently repairs the gap, now in its synthetic functional mode. Unpublished data obtained by the same authors are cited to suggest that polymerase I may synthesize the whole of Col E₁ DNA, unassisted by either DNA polymerase II or III. This role is normally attributed to DNA polymerase III.

he and his colleagues in Canberra had discovered new chemical reactions for extracting and refining copper, and applied for patents in twenty-nine countries for them. If successfully developed to a commercial stage, these reactions could replace the conventional pyrometallurgical process which consumes twice as much energy and emits noxious sulphur dioxide as a byproduct.

In the hydrometallurgical process crude cuprous sulphide is dissolved in an acetonitrile/water solution. On distilling off the acetonitrile, the cuprous ions disproportionate into pure copper, which is precipitated, and cupric ions, which take part in the first reaction. Pure sulphur, rather than sulphur dioxide, is a byproduct. Dr Parker claimed that a practical process developed from these reactions would be rapid, cheap and environmentally clean. It awaits the development of a pilot plant, and to encourage industry to undertake this and to handle patents, the ANU has formed a company ANUMIN.

CONTRACEPTIVE PILL

Link with Thrombosis

from a Correspondent

It is well recognized that the contraceptive pill should not be used by certain women, for example those with a history of thrombosis, diabetes, liver diseases and raised blood pressure. There has not been such strong evidence for a link between heart attacks in young women and the pill, but several reports have suggested that a link may exist. Radford and Oliver have now published further work which strongly suggests that the pill may be a cause of ischaemic heart disease in young women (*Brit. med. J.*, 3, 428; 1973).

The evidence comes from a comparison of the usage of the pill in young women with heart disease with that in the general population. Admissions to the coronary care unit of the Royal Infirmary of Edinburgh were studied over a three-year period and twenty-two women aged between thirty-one and forty-five were diagnosed clinically and biochemically as having had a coronary thrombosis. Six of these (27%) had been taking the pill at or just before the occurrence of the attack, and of the nine in the age group thirty-one to forty, in which coronary thrombosis in women is a fairly rare occurrence, five had been taking the pill (55%).

This compares with figures from various studies which indicate the usage of the pill in the general population to be between 13 and 17%. But most users are below the age of thirty, and the authors estimate a usage rate of 8.3% in women aged between thirty and forty-five and 10.8% in women aged thirty to thirty-nine. So Radford and Oliver

say that the figures which they obtained for the proportion of pill-takers among the women admitted with thrombosis—27% and 55% in the two age groups—are significantly greater than expected.

The main thrust of the article, however, is to support the view that women who already have any likelihood of developing coronary thrombosis are best advised not to take the pill. The authors found that each patient had at least one factor recognized as predisposing them to coronary thrombosis, and the average number of factors was 2.6. Some of these factors, such as increased blood pressure and obesity, can be easily checked in a routine examination, but some of the other factors, raised serum cholesterol levels for example, are not normally looked for in the routine examination which precedes a prescription for the pill. So one of the recommendations that follows from this work is that these more sophisticated tests should be carried out in prospective users of the pill with a family history of coronary thrombosis occurring at an early age.

One curious finding was that of the four women between thirty-one and forty not on the pill yet who developed

coronary thrombosis, three had been sterilized. Removal of the ovaries is known to affect blood cholesterol levels, but in these three cases the ovaries were intact, sterilization having been effected by removal or ligation of the Fallopian tubes. Two of these women had no known risk factor for coronary thrombosis. As the authors point out, this association between sterilization and coronary thrombosis may well be a chance finding. But as sterilization is sometimes recommended as an alternative to the pill for women seemingly at risk from coronary thrombosis, this finding deserves further investigation.

PROTONS

300 GeV Disappoints

from a Correspondent

WITH the first of the very high energy proton synchrotrons coming into operation at the National Accelerator Laboratory, near Chicago, it would be surprising if nuclear physicists were not in for some surprises. Naturally the main interest is the study of the interactions and properties of elementary

Decoordination of RNA and Protein Synthesis

In *Nature New Biology* next Wednesday (September 12) Foury and Goffeau show that RNA synthesis in yeast, like that in bacteria, responds to inhibitors of protein synthesis. In yeast cycloheximide, among other antibiotics, specifically inhibits protein synthesis; chloramphenicol achieves a similar inhibition of protein synthesis in bacteria. Normally, when growth is restricted in the case of both microorganisms (this may be achieved in a number of ways, for example, deprivation of an energy or nitrogen source) RNA synthesis is reduced. Conversely, in normal growth conditions, the inhibition of protein synthesis with the appropriate antibiotics in yeast, as in bacteria, likewise depresses RNA synthesis.

A combination of the two types of "inhibitory" conditions, however, in both yeast and bacteria, leads to a stimulation of RNA synthesis. The authors show that the apparent increase of RNA synthesis in yeast is not due to increased cellular permeability to the relevant metabolites or decreased RNA degradation.

The mechanism responsible for the increase in RNA synthesis is well understood in the case of bacteria. Stress conditions lead to an accumulation, dependent on protein synthesis, of guanosine tetra- and pentaphosphate

(inhibitors of RNA synthesis). Inhibition of protein synthesis is accompanied by a fall in the cellular concentration of the inhibitors of RNA synthesis and a consequent increase in the level of RNA synthesis.

The similarity in the effects of cycloheximide in yeast and chloramphenicol in bacteria suggests that the downshift conditions might produce the accumulation of an inhibitor of protein synthesis like ppGpp in yeast, but this particular substance has not been detected in eukaryotic cells. An alternative possibility is that cycloheximide stimulates the production of a positive control agent in yeast, for example, cyclic AMP, which is known to stimulate RNA synthesis in shutdown conditions in yeast. The relation, however, between the effects of cyclic AMP and cycloheximide remains speculative.

The common denominator in prokaryotes and eukaryotes seems to be a protein, most likely an enzyme, which is turned over rapidly in the cell (and therefore disappears quickly in the presence of a protein synthesis inhibitor), and which is responsible for either synthesis of a low molecular weight substance that negatively controls RNA synthesis (in prokaryotes) or degradation of a low molecular weight substance involved in positive regulation (eukaryotes?).

particles, but nuclear physicists and chemists are also going to use these machines for studies of the interactions of high energy particles with nuclei and of the wide variety of products formed in these interactions. An experiment described by English and colleagues of Purdue University in *Physical Review Letters* (31, 244; 1973) shows that the interaction of 300 GeV protons with silver nuclei is remarkably similar to that found with 11.5 GeV protons—an unexpected and perhaps slightly disappointing result.

In the experiment silver foils were irradiated in a 300 GeV proton beam from the NAL accelerator. After the irradiation, and without any chemical processing, radioactive products formed in the target were then detected and identified using a γ -ray spectrometer. A high resolution Ge detector was used together with a very detailed computer analysis of the spectra obtained. The targets were counted for between 1 and 3 months following the irradiation. The assignment of the observed γ -ray peaks to specific nuclides was then made on the basis of measured energies and half lives.

From the very complicated spectra observed, twenty-seven different nuclides ranging from ^7Be to ^{106m}Ag were identified and their production cross-section measured. The results were compared with similar measurements using 11.5 GeV protons and the production cross-sections found to be remarkably similar in the two cases for all the nuclides measured. The weighted average of the production cross-sections at 300 GeV to those at 11.5 GeV is 0.91 ± 0.07 .

The mass range of the reaction products includes those from relatively simple nuclear reactions, those of more complex spallation reactions and light fragments. Another recently reported study has also shown that spallation cross-sections for vanadium and cobalt remain constant between proton energies of 11.5 and 300 GeV. The fact that the results from the heavier target, silver, also do not change between 11.5 and 300 GeV indicates that the spectrum of excitation energies does not change significantly between these two incident proton energies. Although it is known that meson production increases markedly in this energy range, the increase is not reflected in the excitation energy of the struck nucleus. This suggests that most of the mesons escape from the nucleus without interacting or significant energy loss.

These results are in contrast to those reported at lower energies where, for example, it is found that between 3 and 29 GeV reactions which require very high excitation become more probable at the higher energy. Evidently this trend does not continue as the incident proton energy is raised still further. But

one is reminded that activation studies do not provide a complete picture of nuclear interactions—for that one must await more detailed and extensive experiments.

NUCLEAR STRUCTURE

Alphas on the Surface

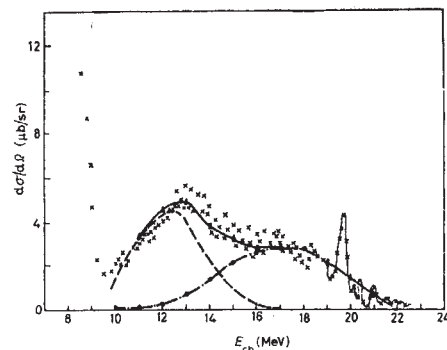
from our Nuclear Theory Correspondent

ADDITIONAL evidence in support of the presence of alpha particles on the nuclear surface has recently been obtained from studies of the energy distribution of the alpha particles emitted when heavy nuclei are bombarded by 17 MeV protons.

A typical spectrum for the nucleus indium-115 obtained by Braga-Marcazan and Milazzo-Colli (*Nuovo Cim. Lett.*, 6, 357; 1973) is shown in the figure. The peaks at the higher energies correspond to alpha particles leaving the residual nucleus in a particular excited state, and the continuum at lower energies corresponds to those emitted from compound nucleus statistical processes.

The statistical evaporation of particles from an excited nucleus and the variation of nuclear temperature with excitation energy are well understood, so that

the energy spectrum of the alpha particles from the compound nucleus can easily be calculated. When this is done the resulting spectrum accounts very well for the alpha particles of lower energies (the dashed curve in the figure), but falls



Energy spectrum of alpha particles emitted at 90° from the interaction of 17 MeV protons with indium-115. $\times$, Experimental data; — — —, statistical theory; and — · —, pre-equilibrium theory. The full line is their sum and is in good agreement with the data.

far short of the intensity found at higher energies.

These alpha particles of higher energy can be understood as coming from

Aleutian Earthquakes Relocated

WHEN a lithospheric plate is overthrust in an island arc region it appears to dip steeply down into the upper mantle and in the duration of ten million years become assimilated. The depth at which submerged plates no longer show their presence by seismicity is anything from 200 to 700 km. The process of turning through 45° or more at the island arc and the shape of the plate after the turn are matters open to considerable doubt. Clearly the region just below the arc is one of high lateral heterogeneity in physical properties, and thus although geophysicists rely very much on earthquakes to tell them both the location of highly stressed regions and the physical properties of the Earth, they are in some difficulty when studying those regions where near the seismic source the physical properties are so disturbed. It is like viewing a point of light through frosted glass and trying to infer both the position of the light source and the properties of the glass.

The Aleutians island arc is an excellent example of this. Nuclear explosions fired on Amchitka are regularly mislocated by tens of kilometres owing to the presence of the underthrust plate. The true location of these explosions is well known, however, so a start can be made

on simulating an upper mantle structure to reproduce the mislocation. This structure looks like a drooping tongue, about 80 km thick and 250 km long with seismic velocity 7% higher in the centre of the tongue than in an "average" upper mantle.

In *Nature Physical Science* next Monday (September 10) Engdahl takes the Aleutians study considerably further. If the structure is indeed of this form, deep earthquakes in the slab can be located more precisely by allowing for this structure in the location process rather than assuming that the Earth is laterally homogenous.

The improved location technique brings all events into a narrow band about 10 km thick. This is an important corroboration of work in other island arcs which has suggested (by different methods) that the region of the dipping plate which has the potential to generate earthquakes is many times narrower than the total thickness of the plate. Engdahl also sees from this careful study a possible correlation between volcanism and deep seismicity. He proposes that volcanism may require the presence of earthquakes at depths of 100 to 125 km. This is a proposal which needs careful examination in other island arcs.

decays taking place before full statistical equilibrium has been established. The model is that the incident proton interacts with an alpha particle on the nuclear surface to form a local "hot spot", or pre-equilibrium excited state consisting of the incident proton, the alpha particle and the alpha hole. This state can decay either by the emission of an alpha particle or by the excitation of more nucleons leading eventually to the full statistical sharing of energy in the compound nucleus. In the case of immediate alpha emission the alpha particles naturally have, on the average, a higher energy than those from the later decay of the compound nucleus, because the temperature of the "hot-spot" is much greater than that of the final compound nucleus. This process of alpha-particle emission is called "pre-equilibrium emission".

The theory of pre-equilibrium emission has been developed by Griffin (*Phys. Rev. Lett.*, **17**, 478; 1966) and others, and enables the energy spectrum of the alpha particles to be calculated. The spectrum found in this way is shown by the dot-dash curve in the figure, and accounts very well for the more energetic alpha particles not given by the statistical theory.

All the parameters of the theory, including the proton-reaction cross-section, the single particle level densities, the excitation energies of the residual and compound nuclei and the matrix element describing the effective two-body interaction, are already known. Only the parameter describing the probability for the incoming proton to strike an already formed alpha particle in the nucleus instead of a nucleon remains to be determined by normalizing the theory of the experimental data. This probability ranges from 0.1 to 0.8 for different nuclei and it will be an important test of the model to see if it can be calculated accurately from the structures of the nuclei concerned.

Subject to this necessary test, this theory gives a plausible account of the origin of the energetic alpha particles emitted from highly excited nuclei, and provides additional evidence favouring the presence of alpha particles on the nuclear surface.

COSMOLOGY

QSO 21-cm Line Found

by our Cosmology Correspondent

THE origin of QSO redshifts remains a contentious issue. But the reported detection of the 21-cm hydrogen line in absorption at a redshift of 0.692154 in the QSO 3C286 suggests that at least 81.5% of the emission line redshift of that object ($z=0.849$) is cosmological.

According to Brown and Roberts, the

most likely origin of this line is in a galaxy lying in the line of sight between Earth and 3C286. They have searched for just such an effect in several QSOs, using the NRAO 300-foot telescope with a receiver tunable over the range 0.75 to 1 GHz. The 3C286 spectrum search revealed a feature at 767.974 MHz, corresponding to a redshifted 21-cm line, first at a bandwidth of 10 MHz and then with 5 MHz and 1.25 MHz bandwidths. A shift of 10 kHz over a period of 15 d, caused by the Earth's motion around the Sun, was also observed (*Astrophys. J. Lett.*, **184**, L7; 1973).

Brown and Roberts "confidently exclude" the suggestion that the line is a recombination feature, because no other such features occur in the interval searched. The position of the line also

rules out a molecular origin in a cloud within our Galaxy. One obvious origin might seem to be in a cloud ejected from the QSO, as suggested by some QSO models (Rees, *Astrophys. J. Lett.*, **160**, L29; 1970). But the narrow velocity dispersion (8.2 km s^{-1}) and evidence that the absorption is occurring in a large column density of neutral hydrogen seem to rule that possibility out too.

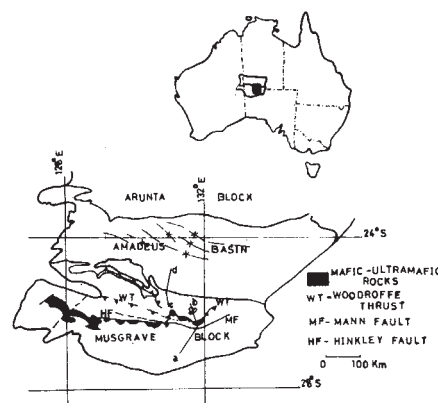
That leaves an intervening galaxy as the only really plausible origin for the line. An isolated discovery of this kind will not set the cosmological world on its heels. Further identifications of this kind, however, could not only resolve the puzzle of the origin of multiple redshifts in QSOs but might also help to determine just how reliable an indicator of distance the redshift is, with all that that implies.

Proterozoic Plate Tectonics in Australia

THE successful application of plate tectonic concepts to the geology of the Phanerozoic has encouraged some Earth scientists to seek evidence of plate activity during the Precambrian. In *Nature Physical Science*, next Monday (September 10), Davidson claims that such searches have concentrated on the Archaeozoic to the neglect of the Proterozoic, although if this is really the case it may simply be because studies of older rocks in the context of plate tectonics are still in their infancy. In any event, Davidson himself redresses the balance to some extent by drawing attention to what he believes is an example of Proterozoic plate tectonics in Australia.

The region concerned is the central Australian complex comprising the Amadeus Basin and the Musgrave Block. From a general comparison of two geological sections (along a-b and c-d in the accompanying map) with the corresponding profiles to be expected from the collision of two continental blocks according to the scheme put forward by Dewey and Bird (*J. geophys. Res.*, **76**, 2625; 1970), Davidson concludes that a collision took place between a proto-Musgrave block and a subducting Arunta block to the north. In the east, the fossil subduction zone appears to correlate with the Woodroffe thrust. The western part of the region lies outside the geological sections studied; but the existence of mafic and ultramafic rocks close to the Hinckley fault suggests that this fault forms the western extension of the fossil subduction zone, being offset to the south from the Woodroffe subduction zone by a short transform fault. Radiometric dating further suggests that the collision and associated events took

place 1,400 million years ago or later (Upper Proterozoic) even though this conflicts with the ages previously assigned to some of the associated rock units on geological grounds.



Although the lithology and structure of the Amadeus-Musgrave complex seem to be largely consistent with a plate tectonic interpretation, there are nevertheless some problems for which only tentative solutions can be put forward at this stage. Two examples are the absence of pillow basalts and pelagic sediments from the overthrust ophiolite sequences and the similar absence of mélangé or blue-schist facies rocks in the subduction zone. Davidson proposes that erosion is responsible for the lack of such rocks, although it follows from this that evidence for their original presence should be found in the sedimentary rocks of adjacent basins. In some cases there is such evidence; but where there is not, it is not yet possible to say whether the original rocks never existed or whether their products have simply not yet been discovered.

ELECTRONICS

Improving Charge-coupled Devices

from our Solid State Physics Correspondent

THE concept of the charge-coupled device (CCD) emerged from Bell Telephone Laboratories about three years ago (Boyle and Smith, *Bell Syst. tech. J.*, **47**, 587; 1970) and several groups have been working hard to bring this very simple but very useful device to fruition. One example was given at a recent European Device Conference (Tompsett *et al.*, *Institute of Physics Conference Series*, No. 15, 174 and 175), where a solid state television camera using a 128×106 element array of these devices was described. Some significant development work is also going on in Britain (and was reported at an Institute of Physics meeting on charge transfer devices held on June 11—see *Nature*, **244**, 11; 1973).

An interesting new concept for operating CCDs has recently emerged from Mead's group at the California Institute of Technology (Mohsen *et al.*, *Appl. Phys. Lett.*, **22**, 172; 1973), where some advanced thinking on device physics and detailed analyses of circuits has indicated that the use of "push clocking" must have many advantages over the conventional "drop clocking", which essentially has to do with the sequence and levels of pulsed voltages applied to the electrodes of the CCD.

As the CCD is not yet widely familiar, a brief description will be given here. The device can be thought of as a line of rectangular metal-oxide-semiconductor capacitors placed edge-to-edge and as close as possible together. Simple theory says that if a voltage is applied to the metal electrode, the silicon energy bands will bend and minority carriers will invade the surface region from the bulk of the semiconductor. In other

words, the potential well at the surface fills up with charge. The higher the applied voltage, the deeper the well and the more charge injected.

The concept is illustrated in Fig. 1, the well being formed in the x - ϕ plane between the oxide surface and some outer plane, which is in fact less well defined than that shown. The situation in a plane at right angles to that of Fig. 1 is shown in Fig. 2(a). By applying different potentials at the surface by means of different plates in the array this potential well can be bound in the z direction. In Fig. 2(a) the electrodes A and C are further away than electrode B but the same well geometry could be obtained if they were coplanar, with different voltages applied. In either situation the result is an electronically bounded and controllable charge packet. The packet can be moved along the chain of electrodes by suitably raising and lowering these potential walls and essentially pouring the packet of charge from one well to the other.

The "conventional" method of moving the packet is to lower one wall of the well, as in Fig. 2(a), allow the charge to move two places (step (1)) and then re-erect the wall behind it (step (2)). The sequence of electric pulses which carries this out can be termed "drop clocking".

The new approach suggested by Mead's group is shown in Fig. 2(b). Instead of lowering a wall the voltages are so arranged that the charge is tipped over the existing wall into the next well. The rising floor of potential well B can be thought of as a piston which pushes the charge over wall C into the next well. Mead and his colleagues call the operation "push clocking" for obvious reasons.

Push clocking has many advantages, the chief of which stems from the fuller use of the power supply voltage available for making the potential wells. The "stroke" of the "piston" in the push clock case (Fig. 2(b)) corresponds to the full 15 V, whereas in the equivalent drop clock case (Fig. 2 (a)) only half the swing is used for the same purpose.

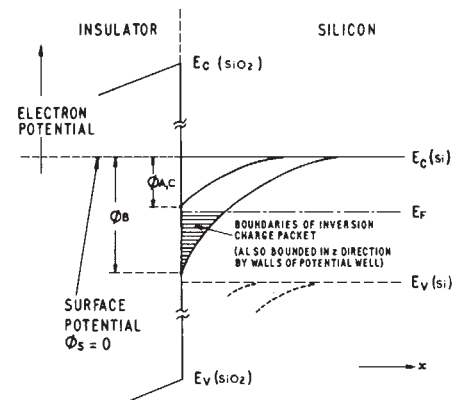


Fig. 1 Concept of the CCD.

The result is that the efficiency of charge transport, which essentially determines the signal-to-noise characteristics of the device, is increased. Also a great impediment to the efficient transfer of charge—the holding up of carriers in interface traps—is ameliorated and the time taken to raise and lower the walls is, as it happens, more efficiently used in shifting the charge. This improves the performance of the device at low frequencies—an important feature. These conclusions by Mead and his colleagues seem to be sound for two-phase clocking. Stanley Brotherton, of the University of Southampton (work to be published) finds, however, that the same advantages do not apply for three-phase clocks. Here, the most important factor for efficient charge transfer is the shape of the rising and falling edges of the clocking pulse waveform.

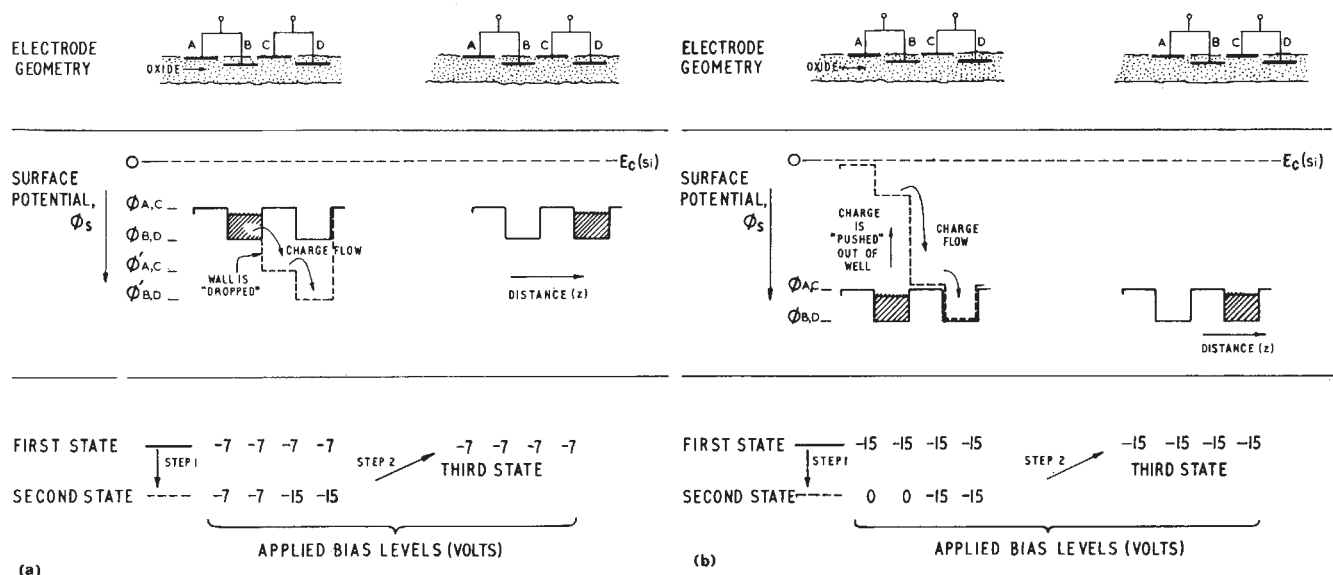


Fig. 2 a, Drop clocking; b, push clocking.

Biosynthesis of Bacteriochlorophyll

R. C. DAVIES, A. GORCHEIN, A. NEUBERGER,
J. D. SANDY & G. H. TAIT

Department of Chemical Pathology, St. Mary's Hospital Medical School, London W2

This article is based on a lecture given by Professor Neuberger at a Symposium on Bio-organic Chemistry held by the Perkin Division of the Chemical Society in association with the Biochemical Society on November 9, 1972.

THE non-sulphur purple bacterium *Rhodospseudomonas spheroides*, which belongs to the group *Athiorhodaceae*, can grow anaerobically in light and aerobically in both light and dark. The concentrations of the three classes of tetrapyrroles, bacteriochlorophyll, haem and vitamin B₁₂, the biosynthetic pathways of which are shown in Fig. 1, are markedly different in cells grown in different conditions of light intensity and oxygen partial pressure¹. In particular, the photosynthetic pigment bacteriochlorophyll is virtually absent from organisms grown at high partial pressure of oxygen in the dark. Light and oxygen both control the formation of these tetrapyrroles, but exactly how is not known. Cohen-Bazire *et al.*² have suggested that light and oxygen control bacteriochlorophyll synthesis by affecting the redox state of one or more of the components of the electron transport chain. Since then much has been learned about the enzymes involved in tetrapyrrole biosynthesis in this organism. Here we describe our recent experiments on a number of these enzymes and discuss possible mechanisms controlling tetrapyrrole biosynthesis.

Aminolaevulinate Synthetase

The decrease observed in the activity of aminolaevulinate (ALA) synthetase (the first enzyme in tetrapyrrole biosynthesis) when a culture of *R. spheroides* was transferred from anaerobic light to aerobic dark conditions, and the increase in activity when a culture was transferred from aerobic dark to anaerobic light conditions, show clearly that this enzyme plays an important role in the control of tetrapyrrole biosynthesis³⁻⁵. The changes in enzyme activity were thought to reflect changes in the amount of enzyme brought about by alterations in the rates of its synthesis and degradation, but recent studies⁶⁻⁹ have shown that the ALA synthetase activity observed in cell-free extracts is influenced not only by the conditions in which the cells are grown, but also by the method of preparing and storing the cell-free extracts.

When a culture of *R. spheroides* growing anaerobically in light was oxygenated, and samples taken at intervals, it was found that the activity of ALA synthetase was high initially and fell by a factor of eight after 1 h oxygenation⁶. The extracts used to assay the activity were prepared as follows. The cells were collected by centrifuging for 15 min at 4° C,

resuspended in buffer and disrupted by sonication. The cell-free extracts were stored for about 1 h at 4° C and then assayed for ALA synthetase activity. When extracts were assayed immediately after cell disruption, however, it was found that oxygenation had produced no apparent effect and the ALA synthetase activity was low in all samples⁶. That is, extracts from anaerobically grown cells activated spontaneously when stored at 4° C in the presence of air, and this activation was reduced, and finally abolished, by oxygenation of the culture.

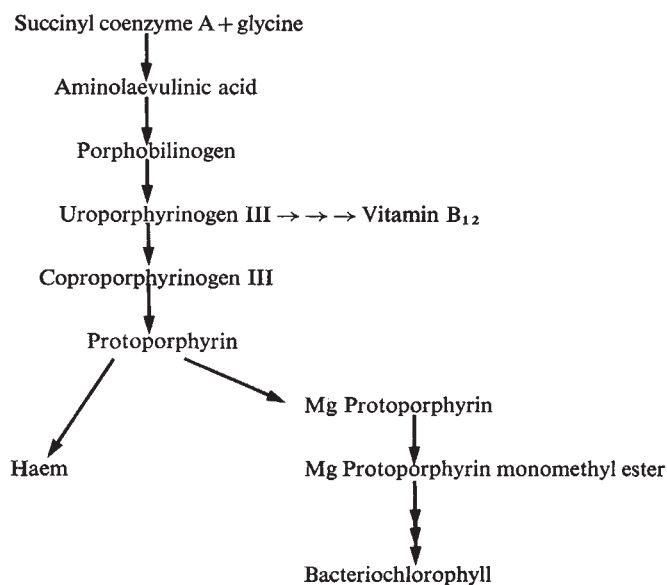


Fig. 1

It seemed surprising that cells growing anaerobically in light and synthesizing bacteriochlorophyll, and cells in which production of bacteriochlorophyll had been stopped by oxygenation² should have the same low activity of ALA synthetase in freshly prepared extracts. It was thought that the low activity in extracts from anaerobically grown cells might be an artefact produced by collection and that the activity in growing cells was more likely to be similar to that obtained in assays with extracts stored at 4° C for 1 h. This indeed was found to be so when samples taken from a culture before and after oxygenation were sonicated without previous collection of the cells¹⁰. The ALA synthetase activity, assayed immediately after disrupting the cells, was high in samples taken before oxygenation and slight in samples taken after oxygenation. From these results it is clear that ALA synthetase may occur in at least two

forms, one with high activity and the other with low activity. The relationship between these two forms of the enzyme has been examined in detail¹¹.

Activation

Spontaneous activation did not occur if cell-free extracts were stored at 4° C anaerobically in the dark, although it did occur anaerobically in the light. Aerobic activation was prevented by cyanide and azide. NADH delayed the activation which started when the NADH had been oxidized. The inhibitory effects of succinate and citrate were prevented by malonate and fluorocitrate respectively. These results suggest that some endogenous compound is probably present in its oxidized state in cells growing anaerobically in the light, but is reduced during collection, which is done anaerobically in the dark.

Activation of ALA synthetase in extracts of oxygenated cells did not occur spontaneously, but could be produced by adding a protein-free fraction prepared from an extract of anaerobically grown cells. This fraction contained, amongst other compounds, sulphur amino acids and glutathione, and the possible role of these compounds in controlling ALA synthetase activity was examined. It was found that the loss of activity of ALA synthetase on oxygenation of the cells was associated with a marked change in the metabolism of low molecular weight sulphur compounds (Fig. 2) as shown by a highly significant change in their concentrations. Amounts of total glutathione and cysteine plus cystine decreased during oxygenation at the same rate as the ALA synthetase activity. Concomitant with the loss of glutathione and cysteine plus cystine a new cellular constituent, homolanthionine, was formed. It has been shown that the disturbance of sulphur metabolism by oxygen results from inhibition of both the reduction of sulphate to cysteine and the con-

0.1 mM to a cell-free extract from oxygenated cells caused a six-fold activation. Activations produced by these compounds required oxygen. The activity of a partially purified preparation of the low activity form of ALA synthetase was not increased by these sulphur compounds, unless a particulate fraction, prepared by centrifuging sonicated cells at high speed, was also added. On further purification of the ALA synthetase, addition of these factors became ineffective. This indicates that the sulphur compounds used do not produce this effect by interacting directly with the enzyme.

Another low molecular weight compound is present in cells grown anaerobically in light which can activate the purified low activity form of ALA synthetase. It seems to interact directly with the enzyme. Activation of ALA synthetase takes place when this compound is added to the enzyme in the presence of all its substrates and cofactors or in the presence of succinate or pyridoxal phosphate alone. When the compound is added to the enzyme in the absence of these substances the enzyme is inactivated. Evidence obtained so far indicates that the activator is a trisulphide of glutathione or cystine. Chromatography of purified preparations of activator on 'Dowex' 50 (H⁺ form) and on phosphocellulose has shown that the elution profiles of activator activity are identical with those of organic polysulphide as estimated by cyanolysis¹⁴. The trisulphides of cystine (thiocystine; bis-(2 amino-2-carboxyethyl)-trisulphide) and of glutathione were prepared. Both these compounds, at concentrations of about 1 μ M, produced a seven-fold activation of ALA synthetase in a cell-free extract from oxygenated cells. As in the case of the activator isolated from cells, both these compounds also activated purified low activity ALA synthetase.

A tentative scheme to account for all these results (Fig. 3) suggests that the activity of ALA synthetase is controlled by the concentration of trisulphides. Their formation and breakdown are regulated by an enzyme dependent sulphhydryl-disulphide couple which is linked to the electron transport chain. The decrease in ALA synthetase activity on collecting cells growing anaerobically in the light is explained by the cells becoming more reduced when they are removed from light². The activator is therefore converted from the trisulphide to the disulphide. On storing the cell-free extracts in the presence of air, the trisulphide is reformed and the ALA synthetase activity increases again. The effect on ALA synthetase activity produced by oxygenating cells seems to be a consequence of the marked decrease in the level of glutathione and cyst(e)ine. Because of this decrease, the cells cannot maintain a high enough concentration of the trisulphide and the activity of ALA synthetase therefore decreases. This proposed mechanism is consistent with the recent demonstration by Tuboi and Hayasaka¹⁵ that partially purified ALA synthetase from *R. spheroides* in its low activity form can be activated by adding cystine and an activating enzyme.

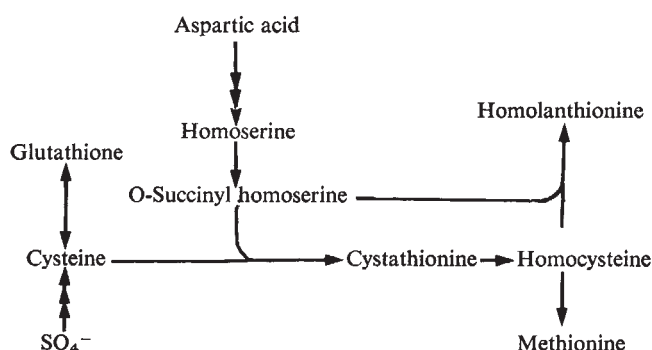


Fig. 2

version of homocysteine to methionine. These effects explain the loss of glutathione and cysteine plus cystine and the accumulation of homolanthionine caused by oxygen. These changes, and the loss of ALA synthetase activity, could be largely prevented by adding threonine or methionine to the culture just before oxygenation. It is known that threonine inhibits the homoserine dehydrogenase of *R. spheroides*¹² and that methionine inhibits the homoserine succinylase of *Salmonella typhimurium*¹³. These inhibitory effects of threonine and methionine may account for their ability to prevent conversion of glutathione and cysteine to homolanthionine on oxygenation (Fig. 2), an assumption which is strengthened by the finding that homoserine reversed the effect of threonine but not that of methionine.

Sulphur-containing compounds also have an effect on ALA synthetase activity *in vitro*. Addition of cysteine, cystine, glutathione and oxidized glutathione to a concentration of

Porphobilinogen Conversion

The enzymatic conversion of porphobilinogen (PBG) to uroporphyrinogen III, the natural isomer, requires two enzymes, PBG deaminase and uroporphyrinogen III co-synthetase. In the presence of PBG deaminase alone, PBG is converted to uroporphyrinogen I, the symmetrical isomer, formally formed by head to tail condensation of four molecules of PBG followed by cyclization. Many mechanisms for the condensation and inversion reactions involved in uroporphyrinogen synthesis have been put forward¹⁶⁻²³. The chief problems are that none of the postulated pyrrolic intermediates such as di-, tri- and open chain tetrapyrroles, has been detected until recently and that chemically synthesized dipyrroles are not readily converted to porphyrinogens when incubated with the enzymes²²⁻²⁵.

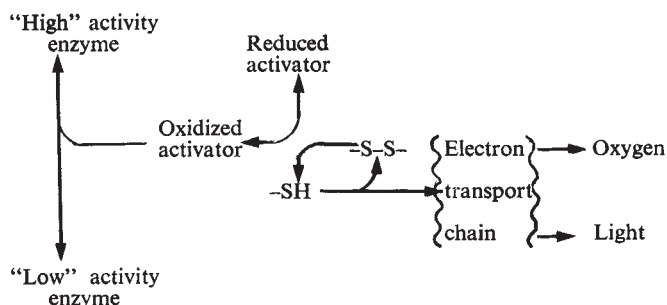


Fig. 3

The results of Llambias and Batlle²¹ show that the enzyme system isolated from soybean callus tissue is different from those isolated from other sources in that it initially consumes PBG faster than it forms uroporphyrinogen. The resulting pyrrolic intermediates have been separated from the reaction mixtures. Preliminary evidence indicates that the intermediates are tripyrroles. The intermediate formed from PBG in the presence of PBG deaminase is converted enzymatically to uroporphyrinogen I on re-incubation with PBG, and that formed from PBG in the presence of PBG deaminase plus uroporphyrinogen III cosynthetase is converted enzymatically to uroporphyrinogen III when reincubated with PBG.

It is also known that high concentrations of hydroxylamine and ammonia inhibit the production of uroporphyrinogen to a much greater extent than the consumption of PBG²⁶. Bogorad's group^{24,27} have isolated compounds formed enzymatically from PBG in the presence of either hydroxylamine or ammonia and have presented evidence that they are a dipyrrole and a tetrapyrrole respectively.

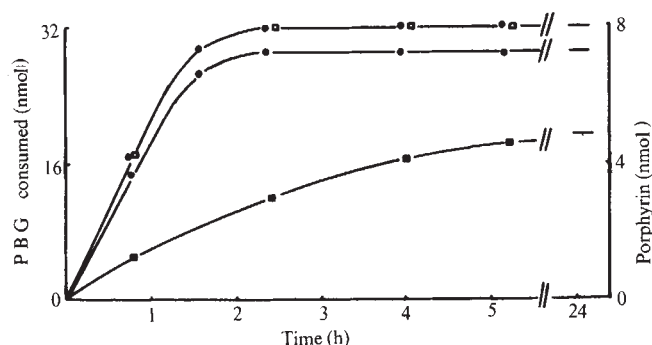


Fig. 4 Porphobilinogen deaminase of *R. spheroides* (purified 200-fold; 6 μg protein ml^{-1}) was incubated with 0.4 mM porphobilinogen in 0.1 M Tris, 5 mM EDTA, pH 8.2, 37° C, in the presence ($\square$, $\blacksquare$) and absence ($\circ$, $\bullet$) of 20 mM hydroxylamine. Samples (100 μl) were analysed for porphobilinogen ($\square$, $\circ$) and uroporphyrinogen ($\blacksquare$, $\bullet$).

In our experiments with PBG deaminase, isolated from *R. spheroides*, the consumption of PBG and the formation of uroporphyrinogen I parallel one another (Fig. 4). In the presence of the base hydroxylamine and also of ammonia or methoxyamine, however, the formation of uroporphyrinogen is much slower than the consumption of PBG, suggesting that pyrrolic intermediates accumulate. After all the PBG has been consumed, uroporphyrinogen continues to be formed at a slow rate.

The intermediates formed in the presence of the three different bases were isolated by gel filtration on 'Sephadex' G25. From their elution pattern and ready conversion to uroporphyrinogen they all seem to be open chain tetrapyrroles. They contain an unsubstituted α -pyrrolic position

and are relatively unstable. The polypyrrole isolated from enzymic incubations performed in the presence of tritiated methoxyamine was radioactive, showing that the base was incorporated into the polypyrrole. These polypyrroles are not acted on by PBG deaminase, nor do they inhibit the enzymatic conversion of PBG to uroporphyrinogen. They are all converted to uroporphyrin I by heating their aqueous solutions at pH 7.5 at 55° C for 1 h, and no free pyrrole remains. In conversion to uroporphyrin, the polypyrrole formed in the presence of ammonia releases about 1 mol of ammonia for every mole of uroporphyrin formed. The compounds formed in the presence of methoxyamine contain about one methoxyamine residue for every four pyrrole units, and release methoxyamine together with a trace of ammonia on heating. The compound formed in the presence of hydroxylamine releases some hydroxylamine but no ammonia on heating²⁸.

It seems likely (Fig. 5) that these compounds are open-chain tetrapyrroles in which the amino group of the amino-methyl side chain of the terminal PBG unit has been replaced by the bases added to the enzymatic reaction mixture. They are presumably formed by reaction of the bases with the enzyme-polypyrrole complex, with subsequent release of the modified product from the enzyme. It is suggested that the enzymatic cyclization step is more susceptible to interference by the bases than are the earlier condensation steps, and that cyclization involves electrophilic attack by a $-\text{CH}_2^+$ group of the pyrrole unit at one end of the open chain tetrapyrrole on the unsubstituted α -position of the pyrrole unit at the other end. The bases are thought to interfere with the cyclization by competing for the $-\text{CH}_2^+$ group.

In experiments with hydroxylamine small amounts of a modified monopyrrole have been found in addition to the modified tetrapyrrole. It seems to differ from PBG in having a hydroxylaminomethyl side chain instead of an amino-methyl side chain.

Coproporphyrinogen Conversion

It is known that ethionine blocks bacteriochlorophyll synthesis in *R. spheroides* and also causes the cells to excrete a large amount of coproporphyrin²⁹. This suggests that methionine is involved in bacteriochlorophyll synthesis. We first showed that the methyl group of methionine was the source of the methyl ester group of bacteriochlorophyll²⁹ and subsequently a particulate enzyme was found which catalysed the formation of magnesium protoporphyrin monomethyl ester from magnesium protoporphyrin and S-adenosylmethionine³⁰. The strong competitive inhibition of this reaction by S-adenosylethionine seemed likely to account for the inhibitory effect of ethionine on bacteriochlorophyll synthesis in whole cells. If this step were the only one requiring S-adenosylmethionine, however, ethionine might have been expected to cause the accumulation of protoporphyrin or magnesium protoporphyrin and not coproporphyrin. More recently, S-adenosylmethionine has also been found to be involved in the metabolism of coproporphyrinogen³¹.

The enzymatic conversion of coproporphyrinogen to protoporphyrinogen involves the oxidative decarboxylation of two of the four propionic acid side chains to vinyl groups. In the mitochondria of liver^{32,33} and in a number of other cells³⁴ the reaction requires molecular oxygen but no other cofactors. An oxygen dependent enzyme catalysing this step was also found in extracts of *R. spheroides*³⁵ but when *R. spheroides* is grown anaerobically in the light, a different reaction not involving oxygen must occur. With cell-free extracts we demonstrated the conversion of coproporphyrinogen to protoporphyrin under anaerobic conditions, when

ATP and methionine or S-adenosylmethionine were added³⁵. Further work³¹ showed the involvement of nicotinamide nucleotides, flavin and non-haem iron. S-Adenosylethionine was a strong inhibitor.

Various mechanisms for the oxidative decarboxylation of the propionic acid side chains have been proposed^{23,36,37}. One is that there is a concerted reaction with no intermediates and another is that a β -hydroxypropionic acid side chain is formed, followed either by sequential dehydration to an acrylic acid side chain and decarboxylation, or by a concerted reaction going directly to the vinyl group. In work with the

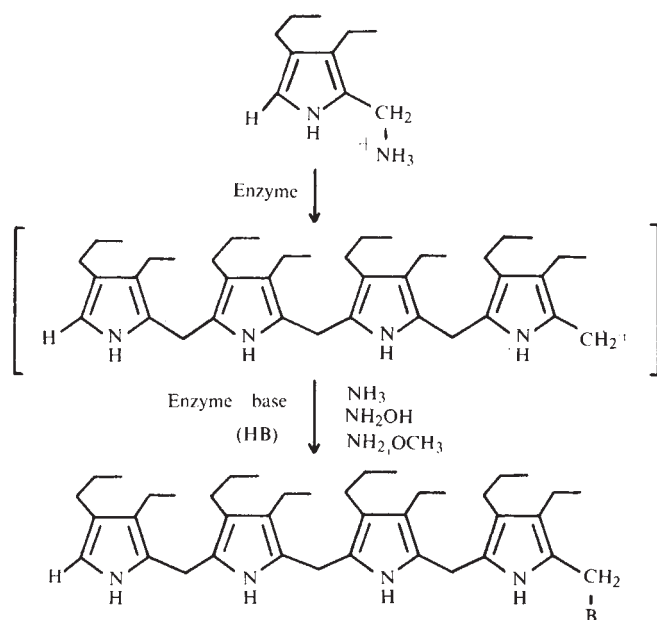


Fig. 5 Enzymatic formation of tetrapyrroles.

aerobic enzyme it has been shown^{38,39} that only one of the two hydrogen atoms on the β carbon atom of the propionic acid side chains is lost, and neither of the hydrogen atoms on the α carbon atom is lost. This evidence is consistent with a concerted reaction either from the propionic acid side chain or a β -hydroxypropionic acid side chain, but is not consistent with the formation of an acrylic acid side chain. The formation of a β -hydroxypropionic acid side chain is possible in the enzymatic reaction involving oxygen but it is much less likely in the anaerobic reaction. Although the exact role of S-adenosylmethionine in the anaerobic reaction is not known, it may be suggested that the positively charged sulphur atom facilitates removal of a hydride ion from the β carbon atom of the propionic acid side chain, thus initiating the concerted dehydrogenation and decarboxylation.

Magnesium Insertion

The enzymatic insertion of magnesium into protoporphyrin has only recently been demonstrated in our laboratory⁴⁰. Whole cells of *R. spheroides* grown semi-anaerobically in the light incorporated magnesium into exogenous protoporphyrin when incubated with EDTA in anaerobic conditions or at low partial pressure of oxygen in the dark. Partial pressures of oxygen greater than 15% inhibited the reaction. The product of the reaction was not magnesium protoporphyrin itself but magnesium protoporphyrin monomethyl ester. If cells were depleted of methionine and S-adenosylmethionine by incubation with ethionine before assaying, then the forma-

tion of magnesium protoporphyrin monomethyl ester was reduced markedly without there being any accumulation of magnesium protoporphyrin. These results indicated that there was a close involvement between insertion of magnesium and methylation of the product, perhaps because a multienzyme complex is responsible for the overall reaction. Although essential for magnesium insertion, methylation was not rate limiting as it was found that formation of magnesium protoporphyrin monomethyl ester from exogenous magnesium protoporphyrin was faster than from protoporphyrin, and was not inhibited by oxygen.

Because of the inability to detect magnesium chelatase activity using cell-free extracts of *R. spheroides*, details of the mechanism of the reaction remain obscure. The effects of a number of inhibitors of hydrogen and electron transport and of energy conservation and transformation reactions, have been tested both anaerobically in the light and at low partial pressure of oxygen in the dark⁴¹. Although we are aware of the difficulties of interpreting results obtained with whole cells, the experiments indicate that electron transport between a b-type and c-type cytochrome together with the coupled synthesis or utilization of ATP is necessary for the chelation of magnesium by protoporphyrin.

Oxygen inhibited the formation of magnesium protoporphyrin monomethyl ester and of bacteriochlorophyll from exogenous protoporphyrin to the same extent⁴¹, but it did not inhibit the conversion of magnesium protoporphyrin monomethyl ester to bacteriochlorophyll. Thus, it seems that the insertion of magnesium is the rate limiting step in the formation of bacteriochlorophyll from protoporphyrin as proposed by Lascelles and Altschuler⁴² and also the only step at which oxygen inhibits the conversion of protoporphyrin to bacteriochlorophyll.

Tetrapyrrole Biosynthesis

As *R. spheroides* makes different amounts of the three tetrapyrroles, bacteriochlorophyll, haem and vitamin B₁₂ when grown in different environmental conditions, it is not surprising that the mechanisms for controlling the intracellular levels of these compounds are not simple. We know nothing about the factors controlling the synthesis of vitamin B₁₂, but we are beginning to learn something about the control of the synthesis of bacteriochlorophyll and haem.

Cells growing anaerobically in the light contain about seventy-five times more bacteriochlorophyll than haem, and about five times more haem than vitamin B₁₂ (ref. 1). Protoporphyrin is the common precursor of haem and bacteriochlorophyll, and if all the protoporphyrin formed can be used by either pathway, then the relative rates of synthesis of these two tetrapyrroles will be governed by the relative rates of incorporation of ferrous iron and magnesium into protoporphyrin¹. At present there is little or no evidence that there are two sets of enzymes forming protoporphyrin for haem and bacteriochlorophyll synthesis respectively. There seem to be two different ALA synthetases⁴³, but it is not known whether there are any differences in their biological functions. *R. spheroides* also has two distinct systems for converting coproporphyrinogen to protoporphyrinogen. One has an absolute requirement for oxygen and the other catalyses an anaerobic reaction. It seems likely that in the cell only one system is active at a time.

Cells of *R. spheroides* grown aerobically in the dark contain only very small amounts of bacteriochlorophyll and they have much lower activities of ALA synthetase, S-adenosylmethionine magnesium protoporphyrin methyltransferase and magnesium protoporphyrin chelatase than have cells grown anaerobically in the light, which contain a large amount of bacteriochlorophyll^{5,40}. On transferring cells from aerobic dark conditions to anaerobic light conditions,

the activities of these enzymes increase markedly over a period of a few hours, and increases are observed before the synthesis of bacteriochlorophyll starts⁵. It is likely that these increases in activity are to a large extent due to synthesis of new enzyme.

Control Mechanisms

We are concerned here, however, with finer control mechanisms. Our main aim is to try to explain the rapid alterations in the rate of synthesis of bacteriochlorophyll which occur in cells when the light intensity and oxygen tension are changed. These alterations are too rapid to be accounted for by induction or repression of enzyme synthesis. On bubbling air through a culture growing anaerobically in the light the synthesis of bacteriochlorophyll stops immediately; on removing the air the synthesis of bacteriochlorophyll starts again immediately². These changes occur without the accumulation of precursors of bacteriochlorophyll¹.

It is clear that the enzymatic insertion of magnesium into protoporphyrin that is the first step after the brach point (Fig. 1), is the rate controlling step in the conversion of protoporphyrin to bacteriochlorophyll. This reaction is markedly inhibited by increasing the oxygen tension. Bubbling air or oxygen through a culture, however, does not lead to a marked accumulation of haem, protoporphyrin or other precursors which strongly indicates that the activity of ALA synthetase is also reduced in these conditions. The inhibition of ALA synthetase may be a consequence of the inhibition of magnesium insertion into protoporphyrin, or the oxygen may independently inhibit ALA synthetase activity, or both mechanisms may operate. Inhibition of ALA synthetase as a consequence of inhibition of the conversion of protoporphyrin to bacteriochlorophyll is the type of control suggested by Lascelles¹. Her work, in which both wild type *R. spheroides* and mutants defective in tetrapyrrole synthesis were grown in a variety of conditions, led her to conclude that an increase in the level of haem, caused by inhibition of magnesium insertion into protoporphyrin, is responsible for the inhibition of ALA synthetase and the non-appearance of intermediates. *In vitro* ALA synthetase of *R. spheroides* is markedly inhibited by very low concentrations of haem and haemin⁴⁴⁻⁴⁶.

Our work concentrates on the control of ALA synthetase by a different mechanism, one not related to the level of haem, but controlled directly by the oxygen tension and light intensity. Although the exact details of this control are not yet known, it would seem that the activity of ALA synthetase is a function of the concentration of an organic polysulphide which probably binds to the enzyme. The concentration of this compound is regulated by the activity of an enzyme dependent sulphhydryl-disulphide couple which in turn is controlled, probably through interaction with the electron transport chain, by the light intensity and oxygen partial pressure. Further details of this control mechanism must await purification of ALA synthetase in both its high and low activity forms, and complete identification of the compound which interacts with it.

From our results it is clear that the original suggestion of Cohen-Bazire *et al.*², that the synthesis of bacteriochlorophyll is controlled by the redox state of one or more of the components of the electron transport chain, is correct. One or more of these components has been shown to be directly involved in the insertion of magnesium into protoporphyrin and in the anaerobic enzyme system which converts coproporphyrinogen into protoporphyrinogen. In addition there is evidence for a chain of reactions linking the electron transport chain to the control of the activity of ALA synthetase.

An interesting feature of tetrapyrrole synthesis in *R. spheroides* is the important part played by sulphur-containing compounds of low molecular weight. The amounts

of glutathione and cyst(e)ine seem to control the activity of ALA synthetase, probably by regulating the concentration of glutathione and cystine trisulphides which activate the enzyme. S-Adenosylmethionine is required for each of the three enzymatic steps leading from coproporphyrinogen to magnesium protoporphyrin monomethyl ester and seven out of the eight methyl groups found in vitamin B₁₂ are donated by methionine⁴⁷. Indeed, disturbances of the sulphur metabolism of this organism have profound effects on the biosynthesis of tetrapyrroles. Although some aspects of sulphur metabolism in this organism are known, much remains to be done.

- ¹ Lascelles, J., *Biochem. Soc. Symp.*, **28**, 49 (1968).
- ² Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y., *J. cell. comp. Physiol.*, **49**, 25 (1957).
- ³ Lascelles, J., *Biochem. J.*, **72**, 508 (1959).
- ⁴ Higuchi, M., Goto, K., Fujimoto, M., Namiki, O., and Kikuchi, G., *Biochem. biophys. Acta*, **95**, 94 (1965).
- ⁵ Gorchein, A., Neuberger, A., and Tait, G. H., *Proc. R. Soc., B*, **171**, 111 (1968).
- ⁶ Marriott, J., *Biochem. Soc. Symp.*, **28**, 61 (1968).
- ⁷ Marriott, J., Neuberger, A., and Tait, G. H., *Biochem. J.*, **111**, 385 (1969).
- ⁸ Marriott, J., Neuberger, A., and Tait, G. H., *Biochem. J.*, **117**, 609 (1970).
- ⁹ Tuboi, S., Kim, H. J., and Kikuchi, G., *Archs Biochem. Biophys.*, **130**, 92 (1969).
- ¹⁰ Neuberger, A., Sandy, J. D., and Tait, G. H., *Biochem. J.* (in the press).
- ¹¹ Neuberger, A., Sandy, J. D., and Tait, G. H., *Biochem. J.* (in the press).
- ¹² Gibson, K. D., Neuberger, A., and Tait, G. H., *Biochem. J.*, **84**, 483 (1962).
- ¹³ Rowbury, R. J., *Nature*, **206**, 962 (1965).
- ¹⁴ Fletcher, J. C., and Robson, A., *Biochem. J.*, **87**, 553 (1963).
- ¹⁵ Tuboi, S., and Hayasaka, S., *J. Biochem., Tokyo*, **72**, 219 (1972).
- ¹⁶ Margoliash, E., *A. Rev., Biochem.*, **30**, 549 (1961).
- ¹⁷ Mathewson, J. H., and Corwin, A. H., *J. Am. chem. Soc.*, **83**, 135 (1961).
- ¹⁸ Bullock, E., *Nature*, **205**, 70 (1965).
- ¹⁹ Cornford, P., *Biochem. J.*, **91**, 64 (1964).
- ²⁰ Dalton, J., and Dougherty, R. C., *Nature*, **223**, 1151 (1969).
- ²¹ Llambras, E. B. C., and Batlle, A. M. del C., *Biochem. J.*, **121**, 327 (1971).
- ²² Battersby, A. R., *Twenty-third Int. Cong. Int. Un. Pure appl. Chem.*, **5**, 1 (1971).
- ²³ Frydman, B., Reil, S., Valasinas, A., Frydman, R. B., and Rapoport, H., *J. Am. chem. Soc.*, **93**, 2738 (1971).
- ²⁴ Pluscec, J., and Bogorad, L., *Biochemistry, N.Y.*, **9**, 4736 (1970).
- ²⁵ Frydman, R. B., Valasinas, A., Rapoport, H., and Frydman, B., *FEBS Lett.*, **25**, 309 (1972).
- ²⁶ Bogorad, L., *Ann. N.Y. Acad. Sci.*, **104**, 676 (1963).
- ²⁷ Radmer, R., and Bogorad, L., *Biochemistry, N.Y.*, **11**, 904 (1972).
- ²⁸ Davies, R. C., and Neuberger, A., *Biochem. J.*, **133**, 471 (1973).
- ²⁹ Gibson, K. D., Neuberger, A., and Tait, G. H., *Biochem. J.*, **83**, 550 (1962).
- ³⁰ Gibson, K. D., Neuberger, A., and Tait, G. H., *Biochem. J.*, **88**, 325 (1963).
- ³¹ Tait, G. H., *Biochem. J.*, **128**, 1159 (1972).
- ³² Sano, S., and Granick, S., *J. biol. Chem.*, **236**, 1173 (1961).
- ³³ Batlle, A. M. del C., Benson, A., and Rimington, C., *Biochem. J.*, **97**, 731 (1965).
- ³⁴ Jacobs, N. J., Jacobs, J. M., and Brent, P., *J. Bact.*, **107**, 203 (1971).
- ³⁵ Tait, G. H., *Biochem. biophys. Res. Commun.*, **37**, 116 (1969).
- ³⁶ Granick, S., and Sano, S., *Fedn. Proc. Fedn. Am. Socs. exp. Biol.*, **20**, 376 (1961).
- ³⁷ Sano, S., *J. biol. Chem.*, **241**, 5276 (1966).
- ³⁸ Zaman, Z., Abboud, M. M., and Akhtar, M., *Chem. Commun.*, 1263 (1972).
- ³⁹ Battersby, A. R., Baldas, J., Collins, J., Grayson, D. H., James, K. J., and McDonald, E., *Chem. Commun.*, 1265 (1972).
- ⁴⁰ Gorchein, A., *Biochem. J.*, **127**, 96 (1972).
- ⁴¹ Gorchein, A., *Biochem. J.*, **134**, 833 (1973).
- ⁴² Lascelles, J., and Altshuler, T., *J. Bact.*, **98**, 721 (1969).
- ⁴³ Tuboi, S., Kim, H. J., and Kikuchi, G., *Archs Biochem. Biophys.*, **138**, 147 (1970).
- ⁴⁴ Gibson, K. D., Matthews, M., Neuberger, A., and Tait, G. H., *Nature*, **192**, 204 (1961).
- ⁴⁵ Burnham, B. F., and Lascelles, J., *Biochem. J.*, **87**, 462 (1963).
- ⁴⁶ Warnick, G. R., and Burnham, B. F., *J. biol. Chem.*, **246**, 6880 (1971).
- ⁴⁷ Scott, A. I., Townsend, C. A., Okada, K., Kajiwar, M., Whitman, P. J., and Cushley, R. J., *J. Am. chem. Soc.*, **94**, 8267 (1972).

Accumulation of Fossil CO₂ in the Atmosphere and the Sea

A. W. FAIRHALL

Institute of Nuclear Sciences, Department of Scientific and Industrial Research, Lower Hutt, New Zealand

Quantitative predictions of the accumulation of fossil CO₂ have important implications for marine life.

THE buildup of fossil CO₂ in the atmosphere due to the exponential increase in the rate of consumption of fossil fuels is a matter of concern. I do not intend here to consider at all the question of possible climatic effects¹; my purpose is rather to present a model which accounts quantitatively for this buildup. Any model, if it is to be useful, must not only account for what has already been observed, but must also make predictions of future trends and/or phenomena not previously observed. In the case of the present model, a significant uptake of fossil CO₂ by the mixed layer of the sea is predicted to have occurred, a prediction at variance with previous models of fossil CO₂ (refs 2-4). Further, the model predicts that this accumulation of fossil CO₂ in the mixed layer may lead to serious problems for the marine biosphere soon after the turn of the century if the present trend of fossil fuel consumption continues unabated.

The key question in any model of fossil CO₂ is what to do about the terrestrial biosphere, which is very complex and not well understood in detail. For example, estimates of the annual fixation of carbon by terrestrial plants range from 10¹⁰ to 10¹¹ ton (ref. 5). Laboratory experiments have demonstrated that some plants fix carbon at a rate proportional to the CO₂ partial pressure⁴. If this were true in general, then land plants would today be fixing CO₂ at a rate 10% greater than a century ago. Fixation by annuals, which are consumed or decay back into CO₂ fairly quickly, is less significant than by long lived species such as trees and humus which can act as semipermanent sinks for fossil CO₂. The quantity of humus formed annually will presumably be related to the rate of photosynthesis, but of the world inventory of humus and its rate of turnover even less is known. In temperate zone forests growth rings vary from year to year, suggesting that factors other than availability of CO₂ limit growth. It seems likely that in tropical forests availability of nutrients, rather than CO₂ supply is growth limiting.

Besides the question of growth rate of trees in response to rising CO₂ levels in the atmosphere, there is the matter of the areal extent of forests. In many areas of the world there is an increasing harvest of mature trees; in other areas forests have disappeared altogether during the past century. It seems very unlikely that the areal extent of forests is today significantly larger than it was a century ago and it is probably less.

The present model is based on the premise that the long lived terrestrial biosphere and humus are not significant sinks for fossil CO₂ emissions. This leaves only the sea as the sink for most of it.

The ability of the sea to accommodate fossil CO₂ is, in theory, severely restricted by the chemical equilibria between hydronium ion, the various inorganic carbon species (CO₂, H₂O, HCO₃⁻, CO₃²⁻), and H₃BO₃ + H₂BO₃⁻. In an equilibrium situation, for a given fractional increase in partial pressure of

CO₂ (P_{CO_2}) the fractional increase in total dissolved CO₂ (ΣCO_2) is only 1/10 as great²⁻⁴ (termed the buffer factor):

$$d\Sigma CO_2 / \Sigma CO_2 = 1/10 (dP_{CO_2} / P_{CO_2}) \quad (1)$$

When this limitation is applied to box models²⁻⁴ of CO₂ in the atmosphere-biosphere-sea, the conclusion is that the amount of fossil CO₂ absorbed into the mixed layer of the sea today is very small. Therefore, in order to account for the quantity of fossil CO₂ which is known to have been produced but which has "disappeared" from the atmosphere, either the sea is turning over very rapidly^{2,4} (on a time scale of less than 200 yr) which is contradicted by the very old radiocarbon "age" of the deep ocean, or most of the fossil CO₂ must be going into the long lived terrestrial biosphere.

In the present model I adopt a different point of view. The sea is observed to be in equilibrium with the ambient atmospheric partial pressure of CO₂ only over a fraction of its surface⁶. Over much of the ocean the sea is either supersaturated, as in the tropical Pacific, or undersaturated, as in much of the North Pacific. The supersaturation of the tropical Pacific can be traced to the upwelling of cold, CO₂-rich water off South America and its drift westwards under the influence of the trade winds. Because today's atmosphere is some 10% richer in CO₂ than that of a century ago, when this water reaches equilibrium with the atmosphere it will be 1% richer in total CO₂, according to equation (1). Integrated over time this represents a significant increase in total CO₂ of the mixed layer over that which it would have had if there were no fossil CO₂ in the atmosphere. So far as the undersaturated areas of the ocean are concerned, the mixed layer can clearly accommodate additional CO₂ and hence is a potential sink for fossil CO₂. This is true today and it must certainly have been true in the past. The model assumes the sea will continue to be a sink for the indefinite future.

Therefore the buffer factor restriction applies only to some fraction (1-x) of the area of the ocean; over the remaining fraction, x, the buffer factor does not apply because the sea is not in equilibrium with the atmosphere. The model predicts that a quantity of fossil CO₂, n_m , has been absorbed by the mixed layer of the sea. The unit (p.p.m.) for measuring fossil CO₂ is chosen to be its volume at STP in units of 10⁻⁶ times the volume of the atmosphere at STP (3.93 × 10¹⁸ m³). In terms of this unit, the natural (pre-industrial) level of dissolved inorganic carbon (total CO₂) in the mixed layer is ~360 p.p.m. Over the fraction, f, of the ocean having a thermocline, the concentration of total CO₂ increases down the thermocline by 10 to 20% because of the redissolution of the rain of detritus in the deep ocean. Thus over this fraction of the ocean there is an upward diffusive flux of dissolved CO₂ species which, with upward advection, balances the downward particulate carbon flux.

The effect of an increasing absorption of fossil CO₂ into the mixed layer would be to perturb (decrease) the concentration gradient in total CO₂ near the surface which means that the upward diffusive flux would be diminished. The decrease in upward flux, which is proportional to n_m , is taken to be formally equivalent to a downward flux ϕ of fossil CO₂ superimposed on the steady state upward flux of natural CO₂.

Taking the perturbation by fossil CO_2 to vanish at the bottom of the thermocline, of average depth T , the perturbed total CO_2 gradient in the thermocline is given by

$$\Delta G = n_m / t A T \text{ p.p.m. m}^{-4} \quad (2)$$

where A is the area of oceans and t is the average thickness of the wind-mixed surface layer ($t \approx 85 \text{ m}$).

ΔG is superimposed on the natural CO_2 gradient and the flux perturbation is

$$\phi = K_z \Delta G f A = (K_z n_m f) / t T \quad (3)$$

where K_z is an average eddy diffusion coefficient which is assessed from models of the thermocline⁷ ($K_z \approx 1 \text{ cm}^2 \text{ s}^{-1} = \pi \times 10^4 \text{ m}^2 \text{ yr}^{-1}$). The quantity (ϕ/n_m) is the fraction of the cumulative amount of fossil CO_2 removed from the mixed layer each year, which is defined as an eddy diffusive transfer coefficient, k_{m-d} (e.d.). Taking $T = 10^3 \text{ m}$ and $f = 0.9$

$$\phi/n_m \equiv k_{m-d} \text{ (e.d.)} = K_z f / t T = 1/30 \text{ yr}^{-1} \quad (4)$$

Although fossil CO_2 is removed from the mixed layer to the deep ocean by advection over the fraction $(1-f)$ of the ocean which is cooled enough to sink to the bottom, there is a return flow up the thermocline over the rest of the ocean which becomes enriched in fossil CO_2 by downward diffusion, until by the time this upward flow reaches the top of the main thermocline it has the same fossil CO_2 concentration as the mixed layer. Hence it returns a quantity of fossil CO_2 to the mixed layer equal to that advected into the deep ocean; so advection results in no net transfer of fossil CO_2 , merely a redistribution in space in the deep ocean. Any fossil CO_2 advected into the deep ocean is expected to remain there for 10^3 yr before returning to the surface.

The differential equations representing the change in fossil CO_2 content of the atmosphere (n_a) and the mixed layer of the sea can now be written as

$$dn_a/dt = P(t) - x k_{a-m} n_a + x k_{m-a} n_m \quad (5)$$

$$dn_m/dt = x k_{a-m} n_a - x k_{m-a} n_m - k_{m-d} \text{ (e.d.)} n_m \quad (6)$$

where $P(t)$ is the annual emission of fossil CO_2 ; k_{a-m} is the transfer coefficient for the exchange of CO_2 between the atmosphere and the sea which is known⁸ from observations on bomb ^{14}C to be around $1/5 \text{ yr}^{-1}$; the corresponding back transfer coefficient from sea to atmosphere, k_{m-a} , is $1/6 \text{ yr}^{-1}$, smaller by a factor of 1.2, the ratio between total natural CO_2 in the mixed layer to that in the atmosphere. The quantity $(k_{a-m} n_a - k_{m-a} n_m)$ represents the net transfer of fossil CO_2 which would occur if fossil CO_2 exchanged between the atmosphere and the mixed layer as freely as natural CO_2 without the buffer factor limitation. According to my hypothesis a net transfer is allowed only over some fraction, x , of the ocean.

Before equations (5) and (6) can be integrated x must be specified. Although this could be treated as an adjustable parameter, I estimated a value as follows. The values of $P(t)$ and dn_a/dt can be specified at one point in time but this still leaves four unknowns: x , n_a , n_m and dn_a/dt . A third equation can be written which expresses the reasonable assumption that the relative rates of increase of n_a and n_m at present are proportional to their respective cumulative amounts;

$$dn_a/dn_m = n_a/n_m \quad (7)$$

The total CO_2 content of the atmosphere at any time is $N_a + n_a$, where N_a is the pre-industrial level of CO_2 in the atmosphere. N_a is uncertain but a value around 292 is usually taken as the best estimate^{1,2,4}. Applying equations (5)–(7) to 1971, when $N_a + n_a = 321 \text{ p.p.m.}$ (ref. 9), and taking $N_a = 292 \text{ p.p.m.}$ ($n_a = 29 \text{ p.p.m.}$), $P(1971) = 2.0 \text{ p.p.m.}$, $dn_a/dt(1971) = 1.0 \text{ p.p.m.}$ and x is 0.30. This is a reasonable estimate for the area of the ocean which is observed not to be in equilibrium with the atmosphere. These values incidentally also predict a value of

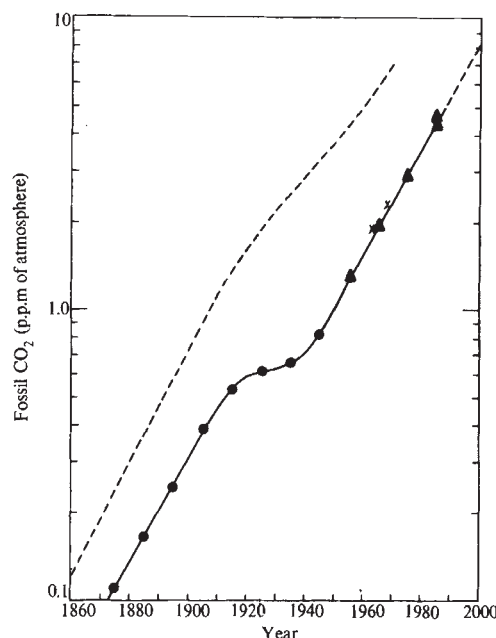


Fig. 1 Annual production of fossil CO_2 since the 1870s (lower curve). ●, From ref. 10; ▲, estimates of OECD, which with the two data from the 1960s are from ref. 11. The upper curve gives the cumulative total to the present day ($\times 1/10$).

14.7 for n_m in 1971. Of course the actual values which n_a , n_m and dn_a/dt will be calculated to have according to the model are a function of $P(t)$, and the other parameters of the model integrated over 140 yr of fossil fuel production, which is as yet unspecified. Therefore estimating x in this way ought not to be construed as having built in the desired results *a priori*.

It is only necessary now to specify $P(t)$ and some initial boundary conditions in order to integrate equations (5) and (6) to see what the model predicts. Estimates^{10–12} of $P(t)$ are shown in Fig. 1. The latest and most detailed estimate is that of Keeling¹², whose unsmoothed data were used in the numerical integration. The back extrapolation of the exponential increase of fossil fuel production in the nineteenth century was truncated at 1830 when $P(1830) = 0.01 \text{ p.p.m.}$ The initial boundary conditions are, then: $n_a = n_m = P(t=0) = 0$ in 1829. The results of the integration are shown in Fig. 2. The predicted values of n_a and n_m in 1971 are 26.4 and 13.4, a little less than the values estimated above. These results depend, however, on the choice of $P(t)$: taking the somewhat larger ($\sim 15\%$) estimates of Revelle and Suess¹⁰ (Fig. 1) the result is $n_a = 33.4$, $n_m = 16.9$, somewhat too large.

Because of the uncertainty in N_a , for comparison of the model results with observations on atmospheric levels of total CO_2 a value of N_a is chosen which gives the observed total CO_2 value of 316.3 in mid-1964 (ref. 9). This then fixes the model predictions at all other times. The predictions of the model since 1958, which marks the beginning of precise measurements, for the two choices of $P(t)$ are superimposed on the observed secular trend in Fig. 3. The fit is quantitative for the case of Keeling's production data but a value of N_a (295.9) slightly larger than the preferred value of 292 is required to produce the fit. On the other hand, the larger production figures of Revelle and Suess give a fairly good fit to observation with a smaller value of $N_a = 290.3$. Clearly, an intermediate level of fossil fuel production would give a good fit to observation with $N_a = 292$. So far as reproducing the secular trend of atmospheric CO_2 is concerned the model could scarcely fit the data better.

Over the longer term, the secular trend in atmospheric CO_2 concentration is less accurately known. But related to it is the "Suess effect", the decrease in the ratio of ^{14}C to total CO_2 in terrestrial plants attributed to dilution of atmospheric $^{14}\text{CO}_2$ by fossil CO_2 . Assuming an unperturbed level of $^{14}\text{CO}_2$ in

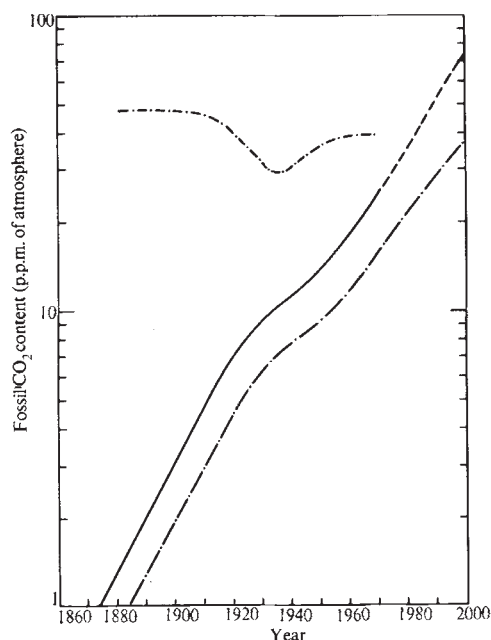


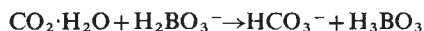
Fig. 2 Model predictions of the cumulative total fossil CO_2 in the atmosphere (n_a) (—) and mixed layer of the sea (n_m) (---). The upper dashed curve gives the ratio of annual increase in n_a to the annual production, expressed as a percentage, using the same numerical scale as the ordinate for this dimensionless quantity.

the atmosphere the Suess effect is given by the ratio $[n_a - n_a(t')]/[N_a + n_a(t')]$, expressed as a percentage, where $t' = 1890$, the date of the ^{14}C reference standard used in ^{14}C dating. The model prediction of the Suess effect is shown in Fig. 4 as the curve drawn through the experimental data¹³⁻¹⁵. The data exhibit variations attributed¹⁴ to variations in the ^{14}C (cosmic-ray) production rate, but the model well reproduces the secular trend.

The model reproduces the observations very well. But it also predicts something not previously recognized: a significant uptake of fossil CO_2 by the sea. This uptake, if real, leads to concern. The reactions by which CO_2 is absorbed into seawater are



and to a lesser extent



Thus the concentration of CO_3^{2-} in the mixed layer will diminish with time as fossil CO_2 is absorbed, until³ the mixed layer will become undersaturated with respect to aragonite and calcite. The sea is presently supersaturated with respect to these minerals, which calcareous organisms form in building their shells. As the sea becomes undersaturated in CO_3^{2-} , the shells of these organisms would tend to dissolve, as would the ocean's coral reefs. The consequences could be very serious for life in the sea.

In order to produce undersaturation of the mixed layer with respect to aragonite, according to Broecker *et al.*³ the CO_3^{2-} concentration would have to be reduced from the present day value of 0.24 mol m^{-3} to 0.06 mol m^{-3} . The quantity of fossil CO_2 required to produce this reduction in the mixed layer can be calculated to be 41 p.p.m. The model predicts that this additional quantity will have accumulated in the mixed layer by about the year 2008 if the exponential growth of fossil fuel continues unabated. By that time the mixed layer will have acquired a fossil CO_2 burden of 55 p.p.m. The model can also tell us the level of fossil fuel emission which could continue indefinitely with the mixed layer remaining just slightly supersaturated (say $n_m = 50 \text{ p.p.m.}$) with respect to

CaCO_3 . In the steady state, removal of fossil CO_2 to the deep ocean would exactly balance the annual emission,

$$P(t) = \text{constant} = k_{m-d}(\text{e.d.})n_m$$

Taking n_m to be 50 p.p.m., $P(t) = 50/30 = 1.7 \text{ p.p.m. yr}^{-1}$. This level of fossil fuel emission was exceeded in 1969. Further, by the turn of the century the annual fossil CO_2 emission is predicted to reach between 7 and 8 p.p.m. yr^{-1} . It seems very unlikely that a large reduction in fossil fuel consumption would occur so that the steady state situation could be achieved unless a dramatic breakthrough in alternative energy sources, in particular controlled thermonuclear fusion, is forthcoming in the very near future.

The model is, of course, just a numerical exercise and before its dire predictions can be taken seriously it will need verification. One test of the model will be the extent to which it continues to predict correctly the future levels of fossil CO_2 in the atmosphere, given the annual production $P(t)$. A better test would be afforded by a careful monitoring of total CO_2 levels in the mixed layer over the next few years. The model predicts an increase in total CO_2 in the mixed layer of about 1.4% in the next decade. Because this is about double the accuracy of present methods for measuring total CO_2 in seawater, this trend, if present, should be detectable within 3 to 5 yr. If it is detected then there will be very little time left to consider what, if anything, could be done about it.

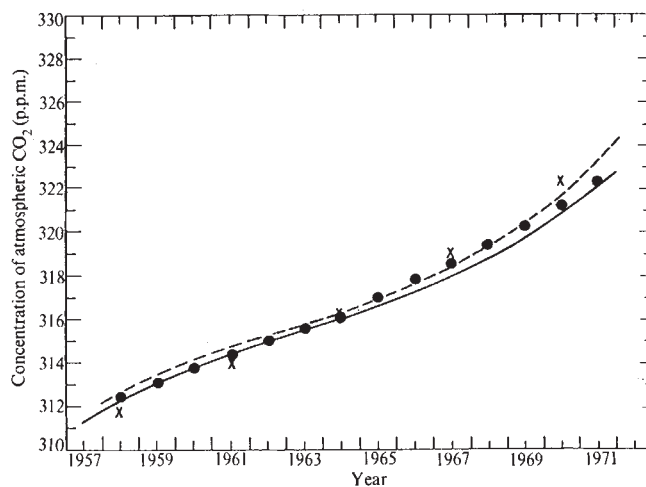


Fig. 3 Fit of the total CO_2 content of the atmosphere since 1958, calculated by the model, to the secular trend as measured at Mauna Loa (---) and Antarctica (—) (ref. 9). ●, Based on Keeling's production data (Fig. 1) and an assumed value of the pre-industrial value of atmospheric CO_2 (n_a) of 295.9; ×, based on the production curve of Revelle and Suess¹⁰ (Fig. 1) and a value of N_a of 290.3.

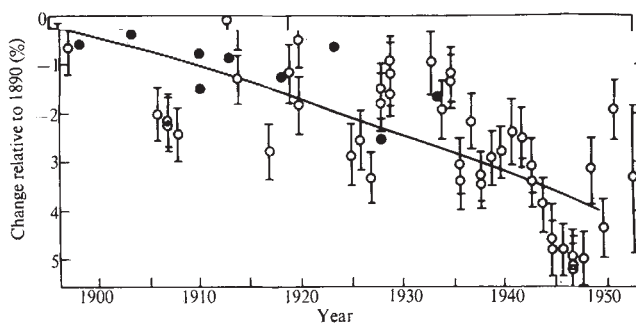


Fig. 4 Decrease in ^{14}C specific radioactivity of tree rings, relative to 1890 wood, attributed to the dilution of atmospheric CO_2 by fossil fuels (the Suess effect). ○, From ref. 5; ●, from ref. 13. Oscillations about the general trend are attributed to variations in the ^{14}C production rate (see ref. 5). After about 1950 these effects are masked by ^{14}C from nuclear weapons.

It would also be of interest to know the response of the calcareous organisms to high ambient partial pressures of CO_2 sufficient to produce undersaturation with respect to CaCO_3 . Two questions require answers: can these organisms build their shells at all under unsaturated conditions; if so, what is the kinetics of the heterogeneous redissolution reaction—can the shells remain undissolved during the lifetime of the organisms? It would be reassuring if the answers to these questions were affirmative.

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¹ Sawyer, J. S., *Nature*, **239**, 23 (1972).

- ² Bolin, B., and Eriksson, E., *The Atmosphere and Sea in Motion* (edit. by Bolin, B.), 130 (Rockefeller Institute Press, New York, 1959).
- ³ Broecker, W. S., Li, Y.-H., and Peng, T.-H., *Impingement of Man on the Oceans* (edit. by Hood, D. W.), 287 (Wiley, New York, 1971).
- ⁴ Keeling, C. D., *Chemistry of the Lower Atmosphere* (edit. by Rasool, S. I.), chapter 6 (Plenum Press, New York, in the press).
- ⁵ Bolin, B., *Scient. Am.*, **223**, 124 (1970).
- ⁶ Keeling, C. D., *J. geophys. Res.*, **73**, 4543 (1968).
- ⁷ Overstreet, R., and Rattray, M., *J. marine Res.*, **37**, 172 (1969).
- ⁸ Young, J. A., and Fairhall, A. W., *J. geophys. Res.*, **73**, 1185 (1968).
- ⁹ Keeling, C. D., Adams, J. A., Ekdahl, C. A., and Guenther, P. R., *Tellus* (in the press).
- ¹⁰ Revelle, R., and Suess, H. E., *Tellus*, **9**, 18 (1957).
- ¹¹ Bolin, B., and Bischof, W., *Tellus*, **22**, 431 (1970).
- ¹² Keeling, C. D., *Tellus* (in the press).
- ¹³ Baxter, W. S., and Walton, A., *Proc. R. Soc.*, **A**, **318**, 213 (1970).
- ¹⁴ Baxter, W. S., and Walton, A., *Proc. R. Soc.*, **A**, **321**, 105 (1971).
- ¹⁵ Cowan, C., Ataluri, C. R., and Libby, W. F., *Nature*, **206**, 861 (1965).

Hypothesis on Differentiation and the Inheritance of Gene Superstructure

P. R. COOK

Sir William Dunn School of Pathology, South Parks Road, Oxford

It is suggested that cellular differentiation is established during development by the development of higher order structural polymorphism of the chromosomes. The differentiated superstructure, once adopted, would be maintained during replication.

It is now widely assumed that nearly all the cells of one organism contain similar genetic information; their DNA contains identical base sequences¹. During differentiation, identical genes are therefore expressed differently in the cells of one organism. In diploid cells the two copies of each gene are probably coordinately controlled and so differences in the expression of the same gene must be studied in different cells. A more critical approach is to consider those situations where homologous DNA molecules, within one cell, behave differently. For example, genes on only one of the two X chromosomes in female cells of eutherian mammals are expressed²⁻⁵.

Because of the success of the ideas of Jacob and Monod^{6,7} in explaining the regulation of bacterial gene expression, differences in the behaviour of identical genes in differentiated cells are usually explained in terms of an association of genes with repressor or activator molecules⁸. I propose an alternative mechanism for maintaining the inactivity of the X chromosome. Differences in the expression of identical base sequences might arise from differences in gene superstructure. The term superstructure will be used to include the secondary and higher order structures that might be superimposed upon the primary base sequence of a nucleic acid. If gene superstructure is an important factor determining gene expression, perhaps superstructures

of DNA, like primary base sequences, may be replicated and inherited. DNA might therefore contain two kinds of heritable information: one kind which is stored in the primary base sequence of a gene, and a second kind which is acquired during development and is contained in its superstructure. The idea is elaborated in a manuscript to be published elsewhere.

Mammalian X Chromosomes

Normal female cells from adult eutherian mammals possess two X chromosomes, for which there is convincing evidence that only one specifies the synthesis of proteins^{2-5,9}. The mechanism that creates the difference in activity acts randomly and so cannot be influenced by particular maternal or paternal base sequences. Differentiation of the two X chromosomes occurs early in development and is heritable: the progeny of a cell in which the maternal X chromosome is inactive have inactive maternal X chromosomes³. When cell hybrids are made by fusion the X chromosomes of the hybrid cell retain the properties they had before fusion^{10,11}. An X chromosome, once inactivated, is not influenced by the presence of additional X chromosomes in the cell and so an acquired characteristic is inherited. Any complete explanation of the inactivation of the X chromosome should account for the stable inheritance of the inactivity of a particular X chromosome. (The creation, *ab initio*, of differences in behaviour of the two X chromosomes will only be of peripheral concern in this discussion (see ref. 5).

"Differential"

The term "differential" is defined as that which constitutes the specific difference between nucleic acids with identical coding properties and which causes their differing behaviour. The differential is recognized by its property of permitting a gene to be expressed. The creator of the differential is

defined as the differentiator. There are two kinds of differential, simple and complex. Identical base sequences may behave differently either because their superstructures are intrinsically different or because they are associated with regulatory molecules in the environment. A difference in gene superstructure would constitute a simple differential; a complex differential depends upon the association of a differentiator with a gene.

The repressor and activator molecules discussed by Jacob and Monod⁶ are examples of differentiators involved in a complex differential. Complex differentials, which depend upon the continued association of differentiators with genes, can be inherited only if the differentiator as well as the target gene is replicated. If the differentiator is a protein then a self-maintaining loop involving the protein, the gene that codes for it, and its target gene must be involved. This is because proteins, unlike nucleic acids, cannot be templates for their own synthesis. In organisms which show complex patterns of differentiation, each pattern might be associated with a specific self-maintaining loop, but it has been argued that this is unlikely¹². Furthermore, small differentiator molecules, being diffusible, should affect all target genes in a cell so that special mechanisms must be invoked to explain the activity of only one of the two X chromosomes in a cell.

The Superstructural Differential

In one cell, two homologous DNA duplexes may be expressed differently because they differ in superstructure. Very little is known about the organization of DNA in chromosomes so that ideas about superstructures are necessarily vague, but these differences may be in base stacking, in sense or degree of supercoiling¹³ or in other forms of higher order structures^{14,15}. Perhaps when DNA is in the B conformation, transcription cannot occur because of the inability of a nascent RNA molecule to adopt this conformation. A gene might be expressed because its DNA exists in the A or C conformation whilst its homologous partner is not, because it is in the B conformation.

A simple form of superstructure is a coiled coil in which tertiary turns are imposed upon the helical turns of the DNA duplex. (In the following discussion two assumptions are made unless stated otherwise. The first is that no covalent bonds are broken. The second is that there is no net rotation of bases about sugar-phosphate bonds in either of the backbone polynucleotide chains of the duplex. This is a plausible assumption because the forces maintaining the double helical structure restrict such free rotation.) Extra supercoils can be maintained in DNA if net rotation about the axis of the helix is restricted, perhaps by the action of histones or by attaching the ends of the duplex to a larger structure, for example, the nuclear membrane. Extrinsic factors restrict rotation in these cases; rotation would be intrinsically restricted in a circle formed by joining the ends of the duplex as is the case in the circular DNA of viruses. (Circles are formally equivalent to linear duplexes in which free rotation of the ends is forbidden.) Additional supercoils in a linear molecule could be maintained intrinsically if the DNA were organized into loops, for example, of the type seen in the lampbrush chromosomes of amphibian oocytes¹⁶.

How might differences in superstructure be created in similar DNA molecules? Superstructures might be introduced after synthesis by coiling the duplex or by cutting one strand of the duplex, introducing superhelical turns into it and then mending the cut. Enzymes that change the superhelical properties of DNA have recently been isolated from both animal and bacterial cells infected with viruses¹⁷⁻¹⁹. Alternatively, superstructures might be introduced during the replication of DNA. For example, if DNA synthesis on one strand occurs in the A configuration and on the other in the C configuration, on subsequent relaxation to the

stable B form, progeny supercoils of opposite sense would be formed.

How might superstructures of nucleic acids be replicated and inherited? Perhaps defined superstructures are re-introduced into the DNA during each cell cycle by the action of diffusible differentiator molecules. This mechanism involves a complex differential; the superstructure, although it controls gene expression, is maintained by a continuing association with differentiator molecules. Alternatively, the replicating machinery of cells might duplicate not only the primary base sequence but also the superstructure of DNA. For example, consider the semi-conservative replication of a circular duplex of DNA proceeding by strand separation and progressive synthesis of daughter strands around the circle. If no covalent bonds are broken, two interlocking circles result, each topologically identical to the parent. (The number of times one circle interlocks with the other is a function of both duplex and superhelical turns.) It is not surprising that superstructure is conserved on replication in this way because, from the point of view of one strand of the parental double helix, all that has been done is to remove a complementary strand and replace it with a newly synthesized strand. The progeny circles can only be freed from one another by cutting covalent bonds to open the duplex. The cut ends must then be mended after separation of the progeny to reform two isolated circles. (Of course, circle opening and closing probably occurs continuously during synthesis.) If net rotation about the axis of the helix is forbidden during opening of the circle, the progeny duplexes remain topologically identical to the parent. It seems likely that during circle opening the cut ends of the duplex will be held in some way, so that they can be rejoined after separation of the progeny. They might be held by the enzymes responsible for making the cut or by some supramolecular organization of the duplex. This holding of the cut ends implies a restriction of rotation about the axis of the helix. If the circle of DNA contained superhelical turns these plausible restrictions ensure that its superstructure is automatically replicated together with its primary base sequence. If supercoils can be maintained in linear DNA molecules they, too, will be conserved on replication in this way.

It has been argued that a simple differential can be automatically replicated and inherited. Once established, a superstructural differential, unlike other differentials, does not require the continued operation of the mechanisms that created it. The idea that development proceeds by a process of directed gene mutation appealed to the early embryologists for the same reason. Specific mutations can be inherited without the operation of specific self-generating loops; so, too, might specific superstructures of DNA. The crux of the problem posed by differentiation concerns the way differentials can be inherited. Superstructural differentials, but not those of other kinds, can be inherited without invoking self-generating loops.

The superstructural hypothesis requires that DNA molecules which have identical base sequences may be polymorphic. We know that the small circular DNA molecules of viruses can adopt a variety of configurations. These molecules contain superhelical turns in addition to the helical turns of the DNA duplex. Intact and closed circular DNA of SV40 can be purified from isolated virions or from cells infected with virus. One strand of the double stranded DNA from these viruses can be cut *in vitro*, and this is followed by a dissipation of superhelical turns; the cut can be subsequently mended by polynucleotide ligase. Intact and circular DNA molecules from SV40 may therefore be obtained from the virions, from cells infected with virus and by making the "relaxed" form *in vitro*. Although these DNA molecules have identical base sequences they differ in superhelical properties²⁰. Parental and progeny SV40 DNA molecules have the same superhelical density, which differs

from that of "relaxed" molecules; a defined configuration is faithfully replicated and inherited during the normal growth of the virus.

Cis and *Trans* Effects

The genes on X chromosomes are coordinately activated or inactivated. Presumably controlling elements on the X chromosome determine the activity of neighbouring genes on the same chromosome (*cis*) but not the activity of genes on the other X chromosome in the same cell (*trans*). What permits these controlling elements to transmit information to genes located *cis* but not *trans*? It is generally believed that information is transmitted to genes located *cis* through polycistronic RNA molecules or through a molecular bridge built from diffusible molecules by cooperative effects. Alternatively, information might be transmitted along a chromosome through the superstructure of DNA. Indeed, a superstructural differential might be expected to lead directly to *cis* effects. The *cis* inactivity of the genes on the X chromosome is correlated with gross condensation of the chromatin to form a Barr body²¹.

How might one distinguish between the effects of the two types of differential? First, if the superstructure of a gene is determined by that of neighbouring DNA, translocation of genes might affect their function; on the other hand, translocated genes should still be subject to regulation by diffusible differentiators. Second, cell fusion mediated by Sendai virus can be used to construct binucleate cells from parental cells with genomes that probably contain identical base sequences but which have different phenotypes. Such studies should provide insight into whether the differentiated phenotype results from *cis* or *trans* phenomena. Third, if superstructures of DNA can be changed, for example by the intercalation of drugs, gene expression governed by a simple differential should be modified. Perhaps the remarkable effects of the thymidine analogue, 5-bromodeoxyuridine, on the expression of differentiated traits result from effects on gene superstructure.

Implications of Heritable Superstructural Differentials

It has been argued that superstructural differentials can be automatically replicated and inherited. Once established, a superstructural differential, unlike other differentials, does not require the continued operation of those mechanisms

that created it. It also provides a simple explanation of *cis* phenomena. Only genes on a chromosome that has adopted a particular superstructure might become inactive; those on other genetic elements might be unaffected. Perhaps heritable differentials have a superstructural basis; other types of differential might have other bases. If so, the superstructure of a gene would be one factor in the hierarchy of mechanisms which govern the expression of the gene. Diffusible differentiators might regulate gene expression within the limits imposed by that superstructure and they would be involved at the creation of the superstructural differential. But the superstructure, once created, would be inherited as an acquired characteristic. The development of an organism would then proceed by an orderly acquisition of gene superstructure.

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- ¹ Gurdon, J. B., and Laskey, R. A., *J. Embryol. exp. Morphol.*, **24**, 227 (1970).
- ² Lyon, M. F., *Phil. Trans. R. Soc.*, **B**, **259**, 41 (1970).
- ³ Lyon, M. F., *Nature new Biol.*, **232**, 229 (1971).
- ⁴ Lyon, M. F., *Biol. Rev.*, **47**, 1 (1972).
- ⁵ Brown, S. W., and Chandra, H. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 195 (1973).
- ⁶ Jacob, F., and Monod, J., *J. molec. Biol.*, **3**, 318 (1961).
- ⁷ Monod, J., and Jacob, F., *Cold Spring Harbor Symp. quant. Biol.*, **26**, 389 (1961).
- ⁸ Britten, R. J., and Davidson, E. H., *Science, N.Y.*, **165**, 249 (1969).
- ⁹ Eicher, E. M., *Adv. Genetics*, **15**, 175 (1971).
- ¹⁰ Siniscalco, M., Klinger, H. P., Eagle, H., Koprowski, H., Fujimoto, W. Y., and Seegmiller, J. E., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 793 (1969).
- ¹¹ Migeon, B. R., *Nature*, **239**, 87 (1972).
- ¹² Ohno, S., *Nature*, **234**, 134 (1971).
- ¹³ Pardon, J. F., and Wilkins, M. H. F., *J. molec. Biol.*, **68**, 115 (1972).
- ¹⁴ Crick, F. H. C., *Nature*, **234**, 25 (1971).
- ¹⁵ Paul, J., *Nature*, **238**, 444 (1972).
- ¹⁶ Gall, J. G., *Brookhaven Symp. Biol.*, **8**, 17 (1955).
- ¹⁷ Alberts, B. M., and Frey, L., *Nature*, **227**, 1313 (1970).
- ¹⁸ Wang, J. C., *J. molec. Biol.*, **55**, 523 (1971).
- ¹⁹ Champoux, J. J., and Dulbecco, R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 143 (1972).
- ²⁰ Eason, R., and Vinograd, J., *J. Virol.*, **7**, 1 (1971).
- ²¹ Barr, M. L., *Expl Cell Res.*, **2**, 288 (1951).

LETTERS TO NATURE

PHYSICAL SCIENCES

Rotation of the Earth's Magnetic Field

HALLEY¹ first noticed that the magnetic declination at a number of sites changed with time in a manner that was consistent with a steady westward drift of the magnetic field relative to the surface of the Earth. After long neglect, interest in westward drift was revived by the work of Bullard *et al.*², who examined the westward drift of the non-dipole part of the field, which they considered to originate less deep within the core than the slower moving dipole field. Since then, there have been numerous other studies (for example refs 3-5) introducing various refinements, such as separation of the field into drifting and standing parts, variation of drift rate with latitude and

separate rates of drift for each harmonic component. A common feature of all such studies is that they consider the drift of the field to be a rotation about the geographical axis. Here we remove this constraint and examine the possibility that the secular changes in the magnetic field might be more closely represented by rotation about an axis other than the geographical axis. This should not be confused with the "northward drift"^{6,7} which is merely the change in the latitude coordinate of the eccentric dipole position.

To avoid spurious effects due to different distribution of data at different epochs, we used the main field models of Malin⁷ which were all based on 5-yr means of observatory data from the same set of eighty observatories for each epoch (1942.5, 1947.5, 1952.5, 1957.5 and 1962.5). These models are sets of spherical harmonic coefficients to degree and order 6 referred to a geographical polar coordinate system (r , θ , λ), where r denotes radial distance from the centre of the Earth, θ denotes colatitude and λ denotes east longitude.



Fig. 1 Position of the pole of rotation of the non-dipole part of the Earth's magnetic field.

The models for two different epochs were transformed to a new coordinate system with its pole at θ' , λ' in the old system^{8,9}. The model for the earlier epoch was then rotated about the new pole by an angle δ such that k , the coefficient of correlation between the non-dipole parts of the two models, was a maximum. A systematic search was made for the values of θ' , λ' and δ that produce the greatest value of k ; that is, for the rotation that reproduces the secular change of the non-dipole field most accurately. The results of such searches are given in Table 1 for all combinations of pairs of models, and also for the more recent models¹² which were similarly based on uniform sets of observatory data for the two epochs 1962.5 and 1967.5.

The pole of rotation has apparently moved from Novaya Zemlya towards the north magnetic pole at a fairly steady rate during the interval considered (see Fig. 1). During this time the drift rate remained near $-0.18 \text{ deg yr}^{-1}$ (the negative sign implies anticlockwise rotation when viewed towards the pole from the centre of the Earth, that is, approximately westwards) but showed a maximum amplitude when the rotation pole was near to the geographical pole.

Although not all the estimates in Table 1 are independent, the regularity of their changes with time strongly suggests that, in general, the pole of rotation differs significantly from the geographical pole. (Had the true pole of rotation coincided with the geographical pole, we would have expected the points

in Fig. 1 to be randomly distributed about the geographical pole, which is clearly not the case.)

Do these results have any physical significance? If the movements of the field reflect corresponding movements of the outer layers of the core² (although Hide¹⁰ has advanced an alternative explanation), the foregoing results imply that the absolute rotation of the outer core is about an axis slightly different from that of the mantle, so that the rotation poles of the core and the mantle will be on opposite sides of the total angular momentum pole (the direction of which is fixed in space) and they will rotate around it with a period very close to 1 d.

For a quantitative investigation, we need to know the rotation rate of the whole core, not just its outer layers. The friction at the core-mantle interface will tend to reduce relative motion between the core and mantle, so it seems likely that the deeper layers will have a greater motion relative to the mantle than that found here. An alternative argument², based on conservation of angular momentum in a convecting core, suggests, however, that westward drift should decrease with depth within the core. As a compromise, we will assume that the rotation found here is representative of the whole core. Because most of the moment of inertia of the core is contributed by its outer layers, this point is probably not critical.

From the 1942.5 to 1962.5 data of Table 1 (representing the longest baseline and hence, probably, the most reliable determination) we may deduce that the angle between the rotation axes of the core and mantle is $1.56 (\delta/T) \sin \theta' = 0.037 \text{ arc s}$, and the difference in rotation periods is $0.66 (\delta/T) \cos \theta' = 0.12 \text{ sidereal s}$. Ignoring lunisolar effects, the Earth as a whole rotates with the period of 1 sidereal d about its angular momentum axis, which is fixed in space. This axis divides the angle between the axes of the core and mantle in the ratio of their moments of inertia, 1:8.5, so that the axis of the mantle would describe a circle of radius $0.004''$ on the celestial sphere once each sidereal day. Its right ascension would be $\lambda' = 4 \text{ h } 11 \text{ min}$ at 0 h Greenwich sidereal time. Ground-based astronomical observations determine the position of the mean rather than the instantaneous axis, so the movements of the mantle axis in space would appear as an apparent diurnal variation of latitude of amplitude $0.004''$. Such a small variation is at the limits of resolution of a photographic zenith tube but should be readily detectable using laser-ranging techniques.

It is interesting to note that the above amplitude is similar to that of the sidereal term found by Fedorov¹¹ in the forced variation of latitude of a perfectly elastic Earth.

Discussion of the changes of the position of the pole of rotation of the magnetic field would be premature until further details of its locus have been obtained from the analysis of longer series of data than those considered here.

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S. R. C. MALIN
I. SAUNDERS

*Institute of Geological Sciences,
Herstmonceux Castle, Sussex*

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Table 1 Pole Positions and Rotation Rates required to give Maximum Correlation between Magnetic Field Models for Different Epochs

Epochs of models	Mean epoch	Interval T (yr)	Pole position		Rotation δ/T (deg yr ⁻¹)
			East longitude, λ' (deg)	Colatitude, θ' (deg)	
1942.5-1947.5	1945	5	65.7	13.7	-0.164
1942.5-1952.5	1947.5	10	64.8	13.0	-0.172
1947.5-1952.5	1950	5	62.8	12.3	-0.180
1942.5-1957.5	1950	15	71.8	9.1	-0.185
1942.5-1962.5	1952.5	20	70.9	7.1	-0.190
1947.5-1957.5	1952.5	10	75.5	7.0	-0.194
1947.5-1962.5	1955	15	68.6	5.1	-0.196
1952.5-1957.5	1955	5	113	2.7	-0.200
1952.5-1962.5	1957.5	10	44	1.7	-0.197
1957.5-1962.5	1960	5	345	3.2	-0.194
1962.5-1967.5	1965	5	263.5	7.3	-0.160

¹ Halley, E., *Phil. Trans. R. Soc.*, **17**, 563 (1693).

² Bullard, E. C., Freedman, C., Gellman, H., and Nixon, Jo, *Phil. Trans. R. Soc.*, **A 243**, 67 (1950).

³ Leaton, B. R., *Roy. Obs. Bull.*, No. 57 (1962).

⁴ Yukutake, T., and Tachinaka, H., *Bull. Earthquake Res. Inst.*, **47**, 65 (1969).

⁵ Richmond, A. D., *RAND Corporation Memorandum*, RM-5191-NASA (1966).

⁶ Nagata, T., and Syono, Y., *J. Geomagn. Geoelect.*, **Kyoto**, **12**, 84 (1961).

⁷ Malin, S. R. C., *Geophys. J. R. astr. Soc.*, **17**, 415 (1969).

⁸ James, R. W., *Geophys. J. R. astr. Soc.*, **19**, 203 (1970).

⁹ Hide, R., and Malin, S. R. C., *Nature phys. Sci.*, **232**, 31 (1971).

¹⁰ Hide, R., *Phil. Trans. R. Soc.*, **A 259**, 615 (1966).

¹¹ Fedorov, Ye. P., *Nutation and Forced Motion of the Earth's Pole* (trans. by Jeffreys, B.) (Pergamon Press, Oxford, 1963).

¹² Malin, S. R. C., and Clark, A. D., *Geophys. J. R. astr. Soc.* (in the press).

Chlorofluorocarbons in the Atmosphere

THE chlorofluorocarbon CCl_3F (trichlorofluoromethane) occurs in the atmosphere¹, and seems especially attractive for use as a tracer of air and water mass movements^{2,3}. Its use as a propellant-solvent for aerosol dispensers results in its entry to the atmosphere where, due to its stability, it can accumulate. There has been an exponential increase in the use of this compound in recent years. Its residence time in the atmosphere has been estimated to be greater than ten years³.

The trichlorofluoromethane is formed in roughly equal amounts with dichlorodifluoromethane (CCl_2F_2) during their production from carbon tetrachloride. In the United States the production rates are 0.12 and 0.18 Mton yr^{-1} for CCl_3F and CCl_2F_2 respectively. The world production rate probably is three times these values. The dichloro-compound is much less sensitive to gas chromatographic assay than its trichloro-counterpart and as yet its occurrence has not been reported in the atmosphere. Because it is somewhat more stable in its packages as an aerosol⁴⁻⁶ and presumably more stable in the atmosphere, one would expect it in higher concentrations than CCl_3F . Further, its relative stability would enhance its use as a tracer. The aim of the investigation we describe here was to seek out the levels of these two chlorofluorocarbons in the atmosphere of Southern California.

Analyses were carried out with a gas chromatograph fitted with an electron capture detector and a column using 'Porapak' T. Air was sampled in brass tubing, either end of which had a "quick connect". Before sampling, the tubes were purged with helium and evacuated.

The air samples were combined with helium to a pressure of about 3.5 atm and subsequently introduced into the gas chromatograph with helium as a carrier gas. Using an electron capture detector, the sensitivity for dichlorodifluoromethane is about 1/45 of that for the trichlorofluoromethane⁷.

Samples were taken from the downtown area of San Diego, the Scripps Institution of Oceanography Pier and the Anza-Borrego desert region, about 100 km north-east of San Diego. The trichlorofluoromethane values are in agreement with those of Lovelock^{1,3} (Table 1) and up to an order of magnitude less than those of the dichlorodifluoromethane.

Table 1 Atmospheric Chlorofluorocarbon Concentrations

		Trichloro- fluoromethane	Dichloro- fluoromethane*
La Jolla pier	Maximum	22.0×10^{-10}	8.0×10^{-9}
	Minimum	0.12	0.3
	Average	3.7 ± 5.6	5.8 ± 4.6
San Diego	Maximum	18.0	7.0
	Minimum	0.1	0.58
	Average	2.9 ± 2.4	3.2 ± 1.4
Desert		0.97	0.70
Marine airs between the United Kingdom and the Antarctic ³		0.5	—
Ireland ¹		0.1 to 2.0	—

* ml per ml of air.

Our expectation that the dichlorofluoromethane would be more abundant than the trichlorofluoromethane is borne out in samples taken from the industrialized area of San Diego and from the adjacent desert regions. The substantially smaller abundance of trichlorofluoromethane is probably related to the relative instability of its C-Cl bonds. The homolytic cleavage of this bond in the presence of such substances as hydrocarbons and alcohols in the atmosphere and in the containers in which it is used can result in its hydrolysis to formic acid by the path⁴⁻⁶



Table 2 Chlorofluorocarbon Concentrations in Laboratory Gases

Gas*	Trichloro- fluoromethane†	Dichloro- fluoromethane†
N_2 (Hi-dry)	6.2×10^{-11}	1.4×10^{-9}
He (99.998%)	2.1×10^{-11}	1.0×10^{-9}
Compressed air	4.0×10^{-10}	3.5×10^{-9}

* All gases were obtained from Liquid Carbonic Company, San Diego, California.

† ml per ml of gas.

Compressed gases, now used in laboratory work, contain a burden of the chlorofluoromethanes (Table 2). The chlorofluorocarbons probably have origins in the airs from which the gases were made, as well as in leakages from refrigeration equipment used in their production. The relatively low value of the trichlorofluoromethane emphasizes its lower stability compared to its dichloro counterpart.

Using an estimated world production of the dichlorodifluoromethane of 0.54 Mton yr^{-1} and an atmospheric concentration of 0.70×10^{-9} ml per ml of air, we obtain a residence time of around 30 yr, which is substantially higher than that of CCl_3F (about 10 yr, ref. 3). This longer residence time in the atmosphere can be of decided advantage in studying air-sea interactions, especially because we have little knowledge about the environmental sinks for either of these chlorofluorocarbons.

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CHIH-WU SU

EDWARD D. GOLDBERG

*Scripps Institution of Oceanography,
La Jolla,
California 92037*

Received July 25, 1973.

¹ Lovelock, J. E., *Nature*, **230**, 379 (1971).

² Lovelock, J. E., *Atmos. Environ.*, **6**, 917 (1972).

³ Lovelock, J. E., Maggs, R. J., and Wace, R. J., *Nature*, **241**, 194 (1973).

⁴ Sanders, P. A., *Soap Chem. Specialties*, **36**, 95 (1960).

⁵ Minford, J. D., *J. Soc. cosmet. Chem.*, **15**, 311 (1964).

⁶ Bohac, S., *J. Soc. cosmet. Chem.*, **19**, 149 (1968).

⁷ Clemons, C. A., and Altshuler, A. P., *Analyt. Chem.*, **38**, 133 (1966).

Pb in Particulates from the Lower Atmosphere of the Eastern Atlantic

MAN-INDUCED changes affecting the trace metal composition of seawater through river runoff have their initial effects in coastal regions close to the source of pollution. It is now recognized, however^{1,2}, that atmospheric transport can introduce pollutants directly to the open ocean. There are still few data on the trace metal compositions of atmospheric particulates from oceanic areas and those that are available³⁻⁵ are from latitudinally restricted locations. Recently, we have carried out a sampling programme⁶⁻⁸ in which particulates have been collected from seawater and from the lower atmosphere (~15 m above the sea surface) over large tracts of the world ocean. The collection details have been described elsewhere⁹ and here we present data on the concentration of Pb in atmospheric particulates collected along the length of the Atlantic Ocean from a variety of air masses. The results are listed in Table 1, and the ship's track is shown in Fig. 1.

Table 1 Average Pb Concentrations in Atmospheric Particulates

Wind system*	Pb concentration (P.p.m., No. of samples, and range)	Enrichment factor†	Potential particulate source area
Westerlies (~50°N~30°N)	1,453 (4; 720-2,000)	113	Western Europe
North-east Trade/Westerlies boundary region (~30°N~20°N)	384 (3; 185-685)	46	Western Europe, North Africa
North-east Trades (~20°N~25°N)	140 (7; 59-238)	12	North Africa, West Africa
ITCZ (~5°N~0°N)	116 (3; 86-142)	10	Central Africa
South-east Trades (~0°S~30°S)	269 (5; 135-490)	23	Angola, South-west Africa
Variable winds off South African coast	695 (7; 162-1,450)	56	South Africa
United States urban air ²		2,300	

* Samples were designated to a particular wind system by latitude, dust loading and dust colour. For example, at 30°N the winds were variable and dusts were grey, even though the area lies within the usual limits of the North-east Trades which are characterized by red-brown dusts.

† See text.

It can be seen from Table 1 that there is a marked decrease in the Pb contents of the particulates southwards from the Westerlies to the Inter-Tropical Convergence Zone (ITCZ) in the northern hemisphere, and a decrease from the variable winds of the South African coast to the ITCZ in the southern hemisphere. This profile offers evidence of anthropogenic effects on the Pb concentrations of particulates from oceanic regions.

In order to evaluate the magnitude of anthropogenic effects on urban air an "enrichment factor", based on the ratio of the concentration of an element to iron in urban particulates, divided by the ratio of the same elements in average crustal material, has been used³. Lead "enrichment factors" calculated in the same way are given in Table 1, together with the average of United States urban particulates. The "enrichment factor" for Pb in the urban air is 2,300 and that of particulates in the Westerlies is 113, that is, the latter is one order of magnitude lower. The Westerlies themselves, however, have an enrichment factor one order of magnitude greater than that of the North-east Trades (12). It may be concluded, therefore, that anthropogenic effects are apparent in the distribution of Pb in marine atmospheric particulates. Further, such effects are geographically dependent, the highest Pb concentrations being found in particulates originating from relatively heavily populated source areas such as Western Europe, which can supply solids to the Westerlies in this region of the North Atlantic³, and South Africa.

No attempt is made here to express the concentrations of Pb in the atmosphere itself because there are considerably more particulates per unit of air in, for example, the North-east Trades than in the Westerlies¹. Because of this any anthropogenic effects in Pb concentrations of the particulates could be masked by the large differences in dust loadings. This question will be considered in detail elsewhere. Here we make the point that Pb concentrations within the particulates themselves vary geographically.

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R. CHESTER
J. H. STONER

Department of Oceanography,
The University,
Liverpool L69 3BX

Received June 7; revised June 29, 1973.

¹ Chester, R., in *Proc. Twentieth Nobel Symp.: The Changing Chemistry of the Sea*, 291 (1972).

² *Baseline Studies of Pollutants in the Marine Environment and Research Recommendations: The IDOE Baseline Conference* (edit. by Goldberg, E. D.) (New York, 1972).

³ Chester, R., and Johnson, L. R., *Nature*, **231**, 176 (1971).

⁴ Hoffman, G. L., Duce, R. A., and Toller, W. H., *Environ. Sci. Technol.*, **5**, 1134 (1971).

⁵ Prospero, J. M., and Bonatti, E., *J. geophys. Res.*, **74**, 3362 (1969).

⁶ Chester, R., Elderfield, H., Griffin, J. J., Johnson, L. R., and Padgugham, R. C., *Mar. Geol.*, **13**, 91 (1972).

⁷ Chester, R., and Stoner, J. H., *Nature*, **240**, 552 (1972).

⁸ Chester, R., Gardner, D., Riley, J. P., and Stoner, J. H., *Mar. Poll. Bull.*, **4**, 28 (1973).

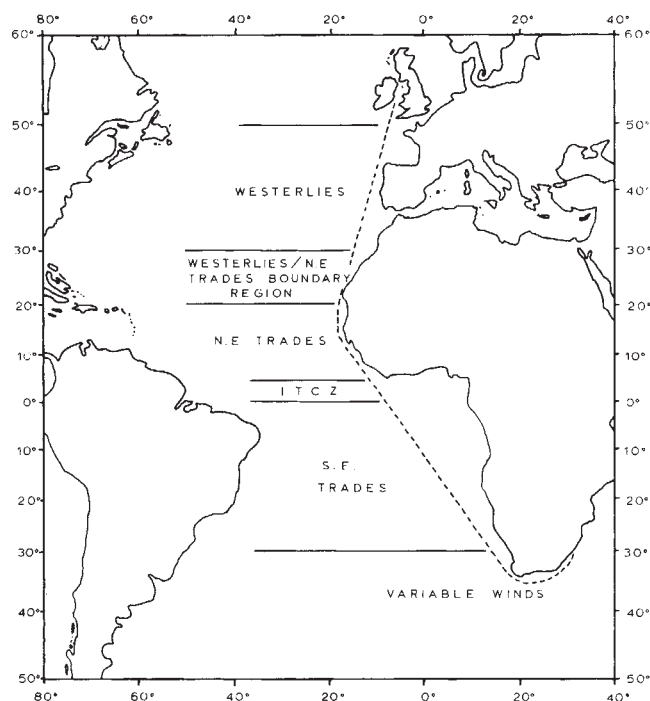


Fig. 1 The ship's track (---) and wind systems in the Eastern Atlantic.

Microorganisms of Carpentarian (Precambrian) Age from the Amelia Dolomite McArthur Group, Northern Territory, Australia

DURING petrological examination of core from DDH Tawallah Pocket No. 1, a diamond drill hole drilled in 1972 by Carpentaria Exploration Co. Pty Ltd (a subsidiary of Mount Isa Mines Holdings Ltd) as part of its exploration activities in the McArthur River region of the Northern Territory, Australia (Fig. 1), N. J. W. C. discovered microfossils. The samples were forwarded to M. D. M. for detailed study, and are briefly described here as the first record of microfossils of their particular age and from this area. The coordinates of the hole are 16° 00' 02" S and 135° 30' 35" E.

Some of the single-celled organisms are clearly similar to *Huroniospora* Barghoorn 1965, described from the 1,900 × 10⁶ yr

old Gunflint Chert of Canada^{1,2}. Although most of the cells in the Amelia Dolomite assemblage have much smaller average diameters, several very close comparisons may also be made between Bitter Springs^{3,4} and Amelia Dolomite forms, even though the time difference between the two formations is probably as much as 700×10^6 yr. As an example, small, usually paired, double-walled cells in the Amelia Dolomite assemblage strongly resemble *Sphaerophycus parvum* Schopf 1968; Schopf regards this species as being a blue-green alga and probably Chroococcacean in affinity. Similarly, a large number of colonial Amelia Dolomite forms could be assigned to the genus *Myxococcoides* Schopf 1968.

One of the most interesting of the colonial bodies in the Amelia Dolomite is a multicellular organism (Fig. 2) which was clearly growing within the sediment in which it is now found. It has a basal attachment, then broadens to a wider multicellular "bulb" which appears to have a rim of rather flattened cells around its margin; it finally thins to a tube from which small branches are given off at irregular intervals. The whole structure is more than 250 μ m high, and its diameter ranges from 25 to 50 μ m. Individual cells range in size from 4.0 to 15.3 μ m and possess the remains of what may originally have been internal cell contents.

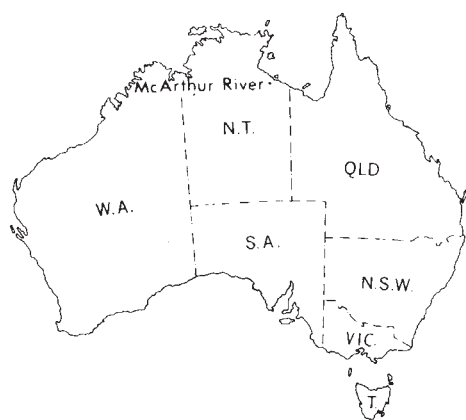


Fig. 1 Locality map.

There appears to be clear cellular differentiation in the colonial structure illustrated in Fig. 3. Two types of cell can clearly be seen—one elongated and arranged in a sub-parallel to radiating manner, and the other type rounded and forming terminations to the elongated cells; the whole colony has a somewhat triangular outline. No such organisms have previously been described from the Precambrian.

The McArthur Group (5.4 km thick) is a carbonate sequence forming part of the type Carpentarian succession⁵ in the McArthur Basin of the Northern Territory, Australia. The group conformably overlies a dominantly arenite succession, 4 km thick (the Tawallah Group), and is in turn unconformably overlain by an arenite-lutite sequence, 3 km thick (the Roper Group). The original stratigraphic relationships in the McArthur Group⁶ have been drastically revised in a new account⁷. The stratigraphic column of the McArthur Group is summarized in Fig. 4.

The lower part of the Amelia Dolomite succession consists of a series of bituminous dolomites and shales, and the microfossils occur at a level 12.3 m above the base. Curved banding in the core could represent stromatolites which are common in the Amelia Dolomite outcrops^{6,7}.

Hamilton and Muir⁸ report the discovery of colonial microorganisms in bituminous matter from the McArthur orebody in the HYC Pyritic Shale Member⁹ of the Barney Creek Formation.

The type Carpentarian sequence is at present the subject of an intensive age determination study; at present the McArthur Group can be placed only within rather broad limits. Granites forming basement to the Tawallah Group are about $1,790 \pm 30$

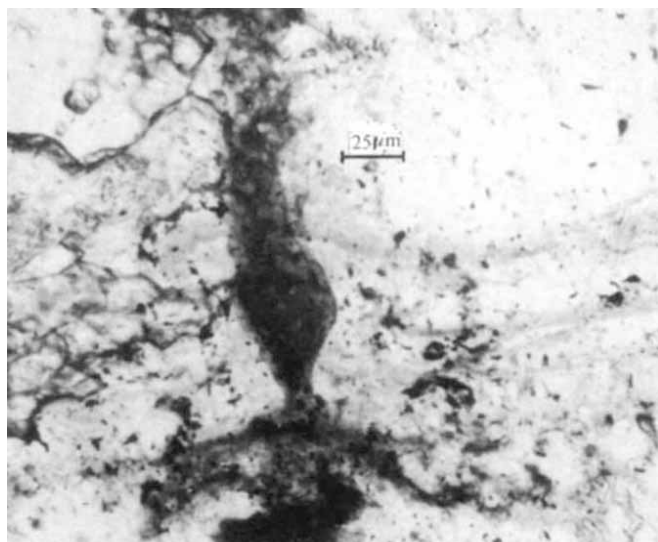


Fig. 2 Large multicellular organism showing basal attachment and position of growth.

$\times 10^6$ yr old, and the Lower Roper Group has a minimum age of $1,390 \times 10^6$ yr (ref. 10). These are the only positive data at present.

The South Nicholson Group, 200 km south-east of McArthur River, overlies the McArthur Group and is thought to be $1,510 \pm 120 \times 10^6$ yr old¹¹.

It seems then, that the age of the Amelia Dolomite is between $1,500$ and $1,800 \times 10^6$ yr; about $1,600 \times 10^6$ yr would seem to be a reasonable prediction at this stage.

Most of the Umbolooga Subgroup (Fig. 4) reflects very shallow water sedimentation in an arid hypersaline environment, alternating, commonly cyclically, between supratidal, intertidal and subtidal. Water depth was greater when the Barney Creek Formation was deposited. The Batten Subgroup represents a carbonate basinal facies with a considerable terrigenous component.

In the Amelia Dolomite itself, superficial comparison of stromatolite forms with modern types¹² may suggest an intertidal environment. The cores, however, show micro-alternations of organic matter and carbonate (sometimes replaced by chert) which closely resemble Recent supratidal deposits in the Persian Gulf (sabquha), and others in the geological past, which are a product of a hot dry climate and hypersaline marine conditions with frequent drying out of the sediment.

The microfossils described here help to fill a gap in the knowledge of Precambrian microorganisms. Stratigraphically,

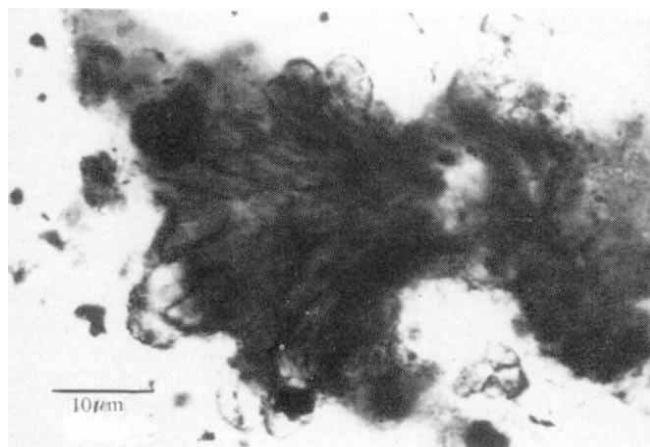


Fig. 3 Multicellular organism showing differentiation of cells with some subparallel elongated cells terminated by smaller spherical cells.

they lie between the well known Gunflint Chert and Bitter Springs Formation assemblages; morphologically, they represent a considerable evolutionary advance on the Gunflint Chert microorganisms. The colonial organisms are the oldest so far discovered and the multicellular organisms show clear evidence of cellular differentiation of a type not observed in present day prokaryotic organisms. The two types of organism

J. JANECEK

Carpentaria Exploration Co. Pty Ltd,
PO Box 321,
Bathurst, NSW

M. D. MUIR

Geology Department,
Royal School of Mines,
Prince Consort Road,
London SW7

K. A. PLUMB

Bureau of Mineral Resources,
Box 378,
Canberra, ACT 2601

Received April 23; revised July 5, 1973.

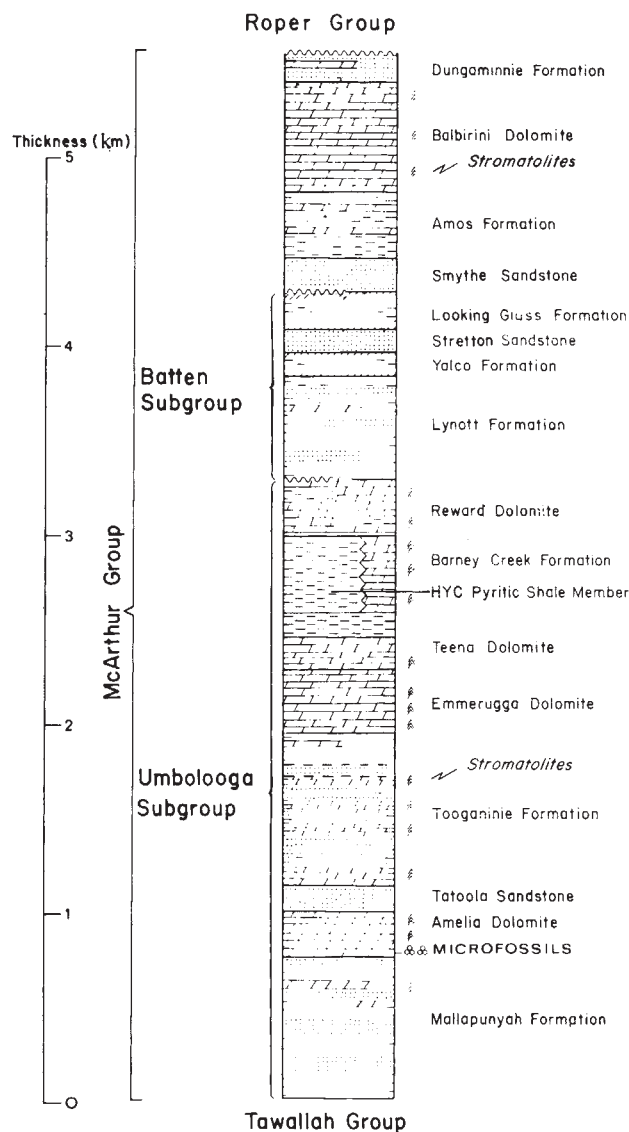


Fig. 4 Schematic column of the stratigraphy of the McArthur Group at McArthur River.

shown in Figs 2 and 3 have large cells and a high level of organization, and share many characteristics in common with the higher eukaryotic algae.

The wide variety and large numbers of microorganisms in the Amelia Dolomite will repay further intensive study, and represent an exciting advance in our knowledge of Precambrian microbiotas.

The Carpentaria Exploration Co. Pty Ltd supplied the drill core on which this communication is based. Conzinc Riotinto of Australia Ltd provided a grant to the Baas Becking Geobiological Laboratory for one of us (M. D. M.) to visit the area. We thank Mr Haddon F. King for his interest and support.

N. J. W. CROXFORD

Mount Isa Mines Pty,
Mount Isa,
Queensland

- ¹ Muir, M. D. *Origins of Life, Proc. Fourth Int. Conf. Origin of Life* (in the press).
- ² Barghoorn, E. S., and Tyler, S. A., *Science, N.Y.*, **147**, 563 (1965).
- ³ Schopf, J. W., *J. Paleont.*, **42**, 651 (1968).
- ⁴ Schopf, J. W., and Blacic, J. M., *J. Paleont.*, **45**, 925 (1971).
- ⁵ Dunn, P. R., Plumb, K. A., and Roberts, H. G., *J. geol. Soc. Aust.*, **13**, 593 (1966).
- ⁶ Smith, J. W., *Bur. Miner. Res. Aust. Explan. Notes*, SE/53-3 (1964).
- ⁷ Plumb, K. A., and Brown, M. C., *Bur. Miner. Res. Aust. Bull.*, **139**, 101 (1973).
- ⁸ Hamilton, L. H., and Muir, M. D., *Mineralium Deposita* (in the press).
- ⁹ Croxford, N. J. W., and Jephcott, S., *Proc. Australas. Inst. Min. Metall.*, **243**, 1 (1972).
- ¹⁰ McDougall, I., Dunn, P. R., Compston, W., Webb, A. W., Richards, J. R., and Bofinger, V. M., *J. geol. Soc. Aust.*, **12**, 67 (1965).
- ¹¹ Compston, W., and Arriens, P. A., *Can. J. Earth Sci.*, **5**, 561 (1968).
- ¹² Logan, B. W., Rezak, R., and Ginsburg, R. N., *J. Geol.*, **72**, 68 (1964).

Depositional Histories of Sand Grains from Surface Textures

BROWN¹ has drawn attention to the occurrence of features of "glacial origin" on sand grains from freshly weathered granite, from beaches, and from streams, all presumably lacking evidence of previous glacial history. We would like to clarify certain misleading statements presented by Brown¹, not with the purpose of discrediting her findings but rather to argue with the interpretation of her data with respect to previously published literature on this subject. Krinsley and Donahue's transmission microscope work² of 1968, referred to by Brown, has been greatly expanded on by much detailed work since the use of the scanning electron microscope to examine sand grain surface textures became widespread³⁻⁹. The statement made by Brown, quoting Krinsley and Donahue² "that an assemblage of textures should characterize any one abrasive process, and that no single feature can be considered completely diagnostic," was modified by Margolis and Kennett⁴ to read "... no single surface feature can itself be used to identify the origin of a sand grain, but rather that certain combinations of features are highly diagnostic when statistically significant percentages of the grains in the sample are examined". With regard to Brown's statements on features of glacial origin Margolis and Kennett⁴ have written: "Features that in previous studies have been individually regarded as diagnostic of glacial origin such as large conchoidal fractures and breakage blocks, are also occasionally found on grains from high turbulence beaches and also occasionally on desert sands. When combinations of surface features are examined statistically, however, glacially derived sand grains exhibit unique combinations of characteristics"^{3,6}. The point here is that the technique had advanced beyond presenting individual photographs as evidence of the history of a sand, and now involves a more quantitative treatment of data.

Because Brown does not present any data on the number of grains examined in each sample, their size range, how individual grains were selected for examination, or how the distribution of features varied on individual grains within each sample, it would be difficult to assess how closely these grains resemble those previously described as being of glacial origin. Descriptions and micrographs of grains of non-glacial, primary origin presented by Brown¹ admittedly resemble grains of glacial origin; but they lack striations, which are features fairly common to many quartz sand deposits with a history of glacial action, although not every grain from deposits of known glacial origin necessarily exhibits striations⁴. Brown also overlooks—or at least does not refer to—previously published surface texture work on quartz grains from granitic regoliths and streams. Large variability in surface features has been found on quartz grains from river sands⁴. Most of the river sand grains examined were interpreted as reflecting their previous transportational and diagenetic history from the source area, rather than fluvial processes, resulting very often in a random distribution of features when fifty or more grains from a single locality are examined. Abrasional pitting and fracturing, however, has been noted on grains from rivers and streams with high turbulent energy⁴.

Most quartz grains from schist, gneiss and granite regoliths examined have exhibited a large variety of chemical solution and overgrowth features, their expression depending on intensity of chemical weathering^{7,10-12}. Large and small conchoidal fractures, small breakage blocks, and semiparallel step-like fractures have also been described from regolith grains; these mechanical features are perhaps the result of frost action, or possibly expansion of surrounding minerals in the source rock by hydration during chemical weathering^{6,7,9}. Quartz grains derived from unweathered regolith material and glacial grains therefore have many fracture and cleavage features in common. Fresh, chemically unweathered grains, however, are very rare, and 90 to 95% of the regolith grains examined to date exhibit varying degrees of complex solution and precipitation features, together with some fracture features listed above^{7,9-12}. The variability of surface features between individual regolith quartz grains is greater than that of any environment investigated, including river sands; this alone can serve to distinguish these grains from those of glacial origin^{4,7,9}.

The similarity in the features on ice-rafted grains from sub-antarctic deep-sea cores and quartz from fresh granitic regoliths noted by Brown¹, may be due to the protection from chemical weathering of fresh regolith material offered by incorporation in the ice sheet during transport to the deep sea. Indeed, we have found that when grains from beneath the Antarctic ice sheet, from icebergs, and of other unquestioned glacial origin are examined, not every grain exhibits an array of features previously described as being diagnostically glacial⁴. A small but significant percentage of these grains exhibit the wide variation in surface features, including those of chemical origin, which are characteristic of regolith grains. The ratio of regolith to glacially abraded grains within a given glacial sample may vary considerably, being a function of such factors as: type of glacier (alpine, continental, wet base, dry base); source rock type and terrain; and availability of easily erodable materials¹³.

If this represents the true situation, and some grains are glacially transported considerable distances from the source area without any change in surface features, then which of the features found on grains of known glacial origin are actually the result of glacial abrasion processes, and which are produced in the source area prior to glacial influence? This, of course, is extremely difficult to answer, because quartz fractures look similar, regardless of the agent (ice, water, air) in which the fracturing occurs. But glacial abrasion cannot be 100% efficient, and some grains may go through the glacial transport cycle without receiving any new surface features. It is not therefore inconsistent with our data that primary and glacial quartz grains can sometimes exhibit similar surface features.

It has been our experience that the best approach to take in trying to decipher the history of sands by examining their surface textures is to examine from fifty to 100 grains from a sample and record the occurrence of features from each grain⁴. Using this technique we can usually provide useful information on a sand's history, although we have not always been successful in providing a unique environmental interpretation.

Judging from the samples we have examined to date, sand grains of glacial origin yield a characteristic distribution of features, distinctly different from other high energy environments including mud flows, landslides, high energy rivers and pebble beaches^{4-9,14}, although we admit that an individual grain from one of these environments may resemble a given glacial grain, and that we have by no means examined all of the infinite varieties of environments containing quartz sand.

STANLEY V. MARGOLIS

DAVID H. KRINSLEY

*Department of Oceanography and Institute of Geophysics,
University of Hawaii,
Honolulu
and*

*Department of Earth and Environmental Science,
Queens College, CUNY,
Flushing, New York*

Received May 29, 1973.

¹ Brown, J. E., *Nature*, **242**, 396 (1973).

² Krinsley, D. H., and Donahue, J., *Geol. Soc. Am. Bull.*, **79**, 743 (1968).

³ Krinsley, D. H., and Margolis, S., *Trans. N.Y. Acad. Sci., Ser. II*, **31**, 457 (1969).

⁴ Margolis, S., and Kennett, J. P., *Am. J. Sci.*, **271**, 1 (1971).

⁵ Margolis, S., and Kennett, J. P., *Science*, **N.Y.**, **170**, 1085 (1970).

⁶ Margolis, S., and Krinsley, D. H., *Geol. Soc. Am. Bull.*, **82**, 3395 (1971).

⁷ Margolis, S., thesis, Univ. California (1971).

⁸ Nordstrom, C. E., and Margolis, S., *J. sediment. Petrol.*, **42**, 527 (1972).

⁹ Krinsley, D. H., and Doornkamp, J., *Atlas of Quartz Sand Grain Surface Textures* (Cambridge University Press, in the press).

¹⁰ Doornkamp, J., and Krinsley, D. H., *Sedimentology*, **17**, 89 (1971).

¹¹ Fitzpatrick, K. T., and Summerson, C. H., *Ohio J. Sci.*, **71**, 106 (1971).

¹² Schneider, H. E., *Sedimentology*, **14**, 325 (1970).

¹³ Warnke, D. A., *Am. J. Sci.*, **269**, 276 (1970).

¹⁴ Krinsley, D. H., and Donahue, J., *Geol. Mag.*, **105**, 521 (1968).

DR BROWN replies: The discussion by Margolis and Krinsley¹ of my work on quartz grain surface textures² involves no criticism of the principal theme of that work, reservations on the application of electron microscopic examination of surface textures to ancient sediments, so there seems to be no controversy.

I regret that certain more recent references were not quoted extensively; however, I note that the important paper used as the basis of the preceding criticism¹ (Margolis and Kennett³) was indeed included. Unfortunately I have not had the pleasure of reading either Margolis's thesis⁴ or the yet unpublished *Atlas of Quartz Sand Grain Surface Textures*⁵.

In response to my critics' request for sampling statistics: it was not my intention to evaluate the depositional environments of the Pleistocene sands but rather to discuss the application of the technique of electron microscopy to ancient sediments using selected examples. But the following comments on sampling serve to demonstrate an important point.

In the original study⁶ electron microscopy was used to supplement other sedimentological techniques (such as grain size analysis) which had failed to discriminate depositional environments. More than 200 Pleistocene sand grains were examined, selected randomly but chiefly around 250 μ m grain diameter because this is a very dominant mode in the well sorted sands. Of these only fifty grains bore any remotely recognizable depositional surface textures; the remainder had surfaces extensively altered by diagenetic surface solution and reprecipitation effects. Only five grains showed sufficient variety of

textures to be called diagnostic. Aeolian, aqueous and glacial type textures were all represented, either alone or concomitantly.

Margolis and Kennett³ have indeed shown that the most satisfactory and scientific approach to the interpretation of textures occurs when "... statistically significant percentages of the grains in the samples are examined". In modern sedimentary environments (or environments preserved from extensive weathering and abrasion, such as ice-rafted Eocene sediments³) this is a viable proposition. But, modern environments represent the experimental laboratory of the sedimentologist and experiments must relate to the interpretation of the ancient environment. I have suggested that "... the value of any new sedimentological technique lies in its application to the interpretation of ancient deposits where there is no *a priori* knowledge"². Is it still a viable proposition to obtain a statistically significant percentage of grains?

In the case of the Pleistocene sands several transportational processes seem to have acted concomitantly but in varying degrees of importance throughout the succession⁶. To evaluate statistically³ the 50 foot succession of sands in North-east Cheshire alone would involve taking an absolute minimum of ten well selected localities and samples at not more than 10 foot intervals, a minimum of fifty samples. Because of the high degree of surface solution these sand grains have suffered, one would have to examine around 2,000 grains from each sample to obtain fifty reasonably unaltered grains, and even these would possess a variety of textures—aeolian, aqueous and glacial. In all a minimum of 100,000 grains would require examination—a marathon task involving 240 d of electron microscopy alone working at the pace of experienced operators like Krinsley and Margolis⁷. If the technique of electron microscopy is applied to the interpretation of more ancient sands an impracticably vast number of grains may need to be examined. It is tempting, therefore, to use far fewer grains (as I did in my original study⁸) and to draw conclusions from statistically inadequate data. From this one may be forced to conclude that the statistical approach to surface texture interpretation is practical only in relatively modern or very well preserved environments and cannot be extended easily to interpret ancient sediments which is the ultimate aim of the sedimentary geologist.

For comparison with the Pleistocene sand grains I used not only quartz grains from freshly weathered granite but also freshly weathered quartzitic Carboniferous Millstone Grit, and Cretaceous Lower Greensand from a marine environment. It was a Lower Greensand, aqueously abraded grain which I compared² to an ice-rafted grain³ and not granitic regolith material as stated by Margolis and Krinsley¹. Although few grains were examined all the granitic quartz grains were angular with high relief and well fractured. This was true for only some of the Millstone Grit grains but these also showed surface striations, comparable with those demonstrated by Krinsley and Margolis⁷ (Fig. 12) and Margolis and Kennett³ (Plate 3F). Since Millstone Grit was probably one of the chief sources of the Pleistocene sands⁸, how is it possible to distinguish satisfactorily between glacially and non-glacially abraded grains? Since a high proportion of post-Palaeozoic sandstones are also derived from pre-existing sandstones, cannot the same problems arise? This very point is admitted by Margolis and Krinsley¹ but in the ancient deposit, where there is little or no *a priori* knowledge of environment, it is a critical factor.

I have suggested² that more exact evaluation of the physical dynamics of depositional environments is required and also a comparison of the dynamics of different environments. I will go further and suggest that more attention should be paid to the nature of the individual quartz grains and their internal controls on surface texture. I believe that Margolis has already discussed the relationship between V-shaped etch pits and crystallographic axes⁴. It is also necessary to understand the relationships between crystal face and the shape/intensity of percussion pits⁸; crystal face and fractures or striations; the effect of inclusions or impurities on quartz etching, pitting

and fracture and possibly even the effect of trace element chemistry on the hardness of quartz grains and their influence on surface textures.

I hope to discuss critically the significance of some of the above factors elsewhere. Other workers in this field (for example, W. B. Whalley, personal communication) are pursuing similar lines of investigation.

I regret that some of my statements² have been thought misleading by Margolis and Krinsley¹. It is not my intention to either lead or mislead but to suggest caution in the application of electron microscopy to ancient sediments.

JOAN E. BROWN

*Ardvreck, 12 Whitefield,
Rush Green Road,
Lymm, Cheshire*

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¹ Margolis, S. V., and Krinsley, D. H., *Nature*, **245**, 30 (1973).

² Brown, J. E., *Nature*, **242**, 396 (1973).

³ Margolis, S., and Kennett, J. P., *Am. J. Sci.*, **271**, 1 (1971).

⁴ Margolis, S., thesis, Univ. California (1971).

⁵ Krinsley, D. H., and Doornkamp, J., *Atlas of Quartz Sand Grain Surface Textures* (Cambridge University Press, in the press).

⁶ Brown, J. E., thesis, Univ. Manchester (1973).

⁷ Krinsley, D. H., and Margolis, S., *Trans. N. Y. Acad. Sci., Ser. II*, **31**, 457 (1969).

⁸ Frondel, C., *Dana's the System of Mineralogy*, III, seventh ed. (Wiley, New York, 1962).

Mechanism for Vapour Explosions

It is well known that when a hot liquid comes into contact with a cold vaporizable liquid an explosion of considerable violence may occur. This type of explosion is called alternatively a vapour explosion, a thermal interaction or fuel-coolant interaction (FCI), the hot liquid being the fuel and the cold liquid the coolant. These interactions are not the result of chemical change; the energy source is the excess heat in the fuel. Fuel-coolant interactions have been observed between molten tin, indium, steel, aluminium and cold water, in the nuclear field between molten uranium dioxide (the fuel) and liquid sodium (the coolant), and in the chemical industry between liquefied natural gas, LNG (the coolant) and water (the fuel)¹. Witte, Cox and Bouvier², Brauer, Green and Mesler³ and Groenvelt⁴ have given resumés of some proposed mechanisms for FCIs but, as they point out, none of those descriptions is really satisfactory.

Several authors^{1,5-7} have recently discussed a model for the LNG-water interactions based on the theory of homogeneous nucleation⁸. It is proposed that the water heats the LNG to the superheated, metastable state. The violent explosions occur when the LNG reaches the homogeneous nucleation temperature and sudden vaporization occurs. Such a scheme cannot, however, completely account for all FCIs.

First, to transfer sufficient energy from fuel to coolant within the observed time scale of the explosion (a few tens of milliseconds for metal/water explosions) an extremely large contact area between fuel and coolant is needed. Witte, Cox and Bouvier² have shown that in the case of metal/water explosions heat transfer rates three orders of magnitude greater than those that occur during boiling processes are required. This result implies that the contact area must increase by a factor of 10^3 . In the case of LNG-water explosions it is just conceivable that the LNG spreads by a sufficient amount (LNG and water have similar densities) but for metal-water explosions no spreading occurs.

Second, although the homogeneous nucleation temperature of water is about 290° C, we have observed explosions between water and tin at an initial temperature below 290° C. An additional drawback to the superheating model exists in the case of metal-water explosions. Water is notoriously difficult to superheat. For example, experiments with water superheated

by more than 10°C require careful preparation. The water must be boiled for several hours and then kept under vacuum. In industrial explosions, where, of course, the water has not been specially treated, it is unlikely that highly superheated water is produced unless some mechanism in the interaction itself reduces the number of nucleation sites.

Because of the limitations of the superheating model we propose an alternative mechanism and indicate some experimental evidence which supports the model. The same mechanism and similar supporting evidence was first proposed independently by Board, Farmer and Poole⁹, but they performed virtually no calculations. We shall describe our calculations and the experimental evidence for the model in detail later.

We divide the interaction into five stages, the last four of which occur cyclically.

(1) As a result of some triggering mechanism the liquids come into intimate contact and a vapour bubble is formed.

(2) The vapour bubble expands and then collapses as a result of condensation in the subcooled coolant. Because of the density difference between fuel and coolant (and also possibly because a solid crust has formed on the fuel) the bubble collapse is asymmetric and a high velocity jet of liquid coolant is formed, directed towards the fuel.

(3) The jet of liquid coolant penetrates the fuel and, because it has a high velocity, its subsequent disintegration and hence the increase in fuel-coolant contact area is extremely rapid.

(4) As the jet of coolant penetrates and breaks up, heat is transferred from the surrounding fuel to the jet and because the surface area is increasing rapidly the total heating rate also increases rapidly.

(5) When the jet has been heated to a certain temperature it suddenly vaporizes and a high pressure vapour bubble forms. The rapid expansion of this bubble disperses the surrounding fuel into the coolant as a spray of small drops in an analogous manner to the spray of water produced by a depth charge. The process now proceeds from stage (2) again.

We regard stage (1) solely as a means of supplying the initial perturbation which causes the first bubble to form adjacent to the fuel surface. All our calculations for the rest of the model are based on the assumption that this bubble has been formed. From the point of view of controlling the FCI, however, stage (1) is of vital importance. If the triggering mechanism can be suppressed then no interaction occurs. We shall discuss later how this may be done. Because of the cyclic scheme some of the energy released from the fuel during each cycle is fed into subsequent cycles and it is this coupling that enables a small initial perturbation to grow. We note that the model provides a mechanism for increasing the contact area between fuel and coolant that is essential to explain the observed rapid heat transfer.

We now give a slightly more detailed description of the model.

Stage (1). The triggering of an FCI may be explained by the collapse of the vapour layer at the fuel-coolant interface. Coolant may then contact the fuel surface, the heat transfer rate is increased at the point of contact and a vapour bubble grows, as required by stage (2). The initial collapse may be brought about in several ways, giving rise to more than one trigger mechanism. For example, Board, Farmer and Poole⁹ have proposed the sudden application of a pressure pulse or, with reference to the boiling curve (Fig. 1), the onset of transition boiling as triggers. In the present experiments, in which small quantities of molten metal are dropped into water, the triggering is caused by cooling to a critical heat flux in the transition boiling regime and provided that this is attained at a fuel temperature above its melting point, an interaction occurs. Any parameter which affects the position of the boiling curve and hence the fuel surface temperature at which the critical heat flux is attained could suppress the interaction completely if the fuel temperature at triggering is below the fuel melting point. The degree of subcooling (that is, the difference between the saturation temperature of the coolant and the

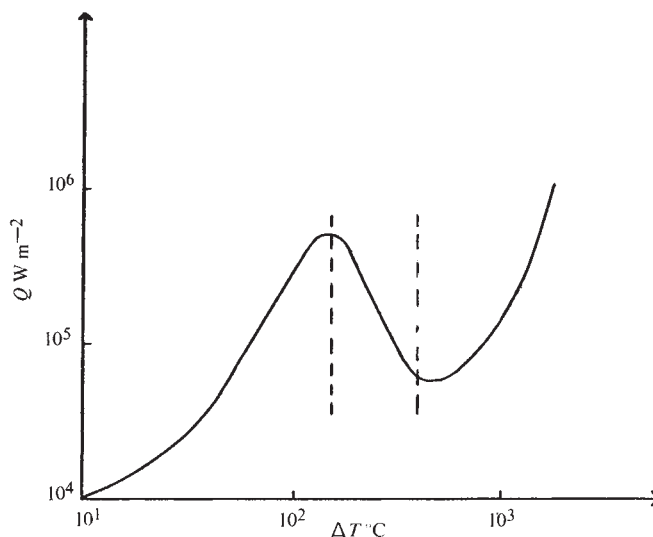


Fig. 1 Typical boiling curve. Q is the heat flux and ΔT the temperature difference between the heating surface and the saturation temperature. The transition boiling regime is between the vertical dashed lines.

bulk coolant temperature itself) is one such parameter and Brauer, Green and Mesler³ have shown that there exists a threshold value for the latter above which no interaction occurs. The ambient pressure should also affect the triggering, for Farber and Scorah¹⁰ have shown that increasing the ambient pressure moves the boiling curve to the left, and consequently should provide a threshold in pressure above which the triggering is suppressed. Of course, even above this threshold pressure, a pressure pulse could still trigger the interaction. Evidence for the application of a pressure pulse collapsing the vapour film has been demonstrated by Board, Farmer and Poole⁹—a mechanical disturbance (in the form of a hammer blow) precipitated an interaction in coolant above the normal threshold temperature.

Stage (2). The initial condition for the i th cycle is a spherical bubble, initial pressure P_i and radius R_i , which expands in the surrounding incompressible coolant of density ρ_c and ambient pressure P_0 ($P_i > P_0$). We assume that the bubble expands without heat transfer until the maximum radius is reached, at which point all the vapour suddenly condenses due to the surrounding subcooled liquid. The resulting cavity then collapses under the external pressure P_0 . Clearly this is an idealized description of this stage—heat transfer does occur during the expansion phase; however, the simple assumptions made here result in an elementary calculation and because, at this stage, we seek only to postulate a possible mechanism the refined calculation is not necessary. The maximum radius and time of expansion can be found using the Rayleigh equation. When an initially spherical cavity collapses adjacent to a solid wall the collapse is asymmetric and a jet of liquid, directed towards the wall, is produced. Plesset and Chapman¹¹ have simulated this situation numerically and they find that the final jet velocity scales as $(\Delta P/\rho_c)^{1/2}$ where $\Delta P (= P_0$ in this case) is the pressure difference causing the collapse. The final jet dimensions scale as the initial (precollapse) cavity radius. Thus the jet dimensions are determined by R_m , the precollapse radius, and consequently by R_i and P_i .

Stage (3). The jet of coolant (diameter d_0 and length L_0) enters the fuel with velocity V_0 . (A solid crust may exist on the fuel surface but Buchanan¹² has shown that provided the bubble radius before collapse is greater than a certain minimum size, penetration will still occur. In these calculations we shall always assume that penetration will occur.) J. P. Christiansen and J. A. Reynolds (unpublished work carried out at the Culham Laboratory) have done a numerical simulation of this situation using the computer code VORTEX¹³. They show that the length of the jet (the calculation is two-dimensional)

increases exponentially with a time constant proportional to d_0/V_0 . Batchelor¹⁴ has investigated the effect of homogeneous turbulence on material lines and surfaces and he also finds that the length of a line increases exponentially. In addition, he has shown that the area of a material surface increases exponentially. While the jet remains liquid its volume is almost constant. Consequently, because the area increases exponentially the average distance between material surfaces must decrease exponentially.

Stage (4). As the jet penetrates the fuel, heat transfer occurs and because the contact area increases exponentially the total heat transfer rate also increases exponentially. Thus the temperature of the jet rises extremely rapidly. Several approximate methods exist for calculating the temperature of the jet. Perhaps the easiest is to assume that the temperature and pressure are uniform within the jet; we have adopted a slight variation of this method in these calculations.

Stage (5). When the jet of coolant has been heated to its saturation temperature it vaporizes provided that nucleation sites are available (heterogeneous nucleation). If heterogeneous nucleation does not occur then vaporization is by homogeneous nucleation at a much higher temperature. We present results for both cases. The saturated vapour and liquid densities are ρ_v and ρ_l and the vapour pressure is P_v . As the density cannot change instantaneously from ρ_l to ρ_v we suppose that this expansion occurs adiabatically. Thus the initial state after vaporization is a high pressure, high density vapour bubble. To define completely the initial state we need the dimensions of the bubble and if we assume that it is spherical with a radius R_i then R_i can be found in terms of the mass of the jet. This high pressure bubble expands and also sends out a pressure wave and because it spends most of its time in the expanded state we shall assume that the Rayleigh equation is valid throughout the motion of the bubble. Thus the vapour bubble is described by stage (2).

We now list some of the consequences of the proposed model. In these calculations water is the coolant and the fuel density is seven times that of water, this being a typical metal density. Because of the cyclic nature of the mechanism a series of pressure pulses occurs and it will be shown in a future paper that the ratio of the peak pressures (measured at a point r from the interaction) of successive pulses is given by

$$\frac{(P_i(r) - P_0)}{(P_{i-1}(r) - P_0)} = \begin{cases} 6.67; & \text{heterogeneous nucleation} \\ 2.90; & \text{homogeneous nucleation} \end{cases}$$

where i is the cycle number. The maximum radius of the bubbles when they expand (stage (2)) is determined by P_0 , the external pressure, as well as by the initial pressure. We find that as P_0 increases the strength of the interaction decreases. Indeed, there exists a threshold value for the external pressure above which any initial perturbation is damped out. This threshold pressure, P_{th} , is given by

$$P_{th} = \begin{cases} 67.5 \text{ bar}; & \text{heterogeneous nucleation} \\ 13.0 \text{ bar}; & \text{homogeneous nucleation} \end{cases}$$

It can also be shown that about 44% of the energy of the bubble is fed into kinetic energy of the jet for the next cycle, thus the process is sustained with high efficiency.

Substantial experimental evidence for such a mechanism has been obtained both by ourselves and Board, Farmer and Poole⁹. By taking high speed cine film of the interaction it may be seen directly that the interaction proceeds in a series of cycles. In addition, examination of the debris remaining after an interaction indicates that explosions have occurred from within small lumps of fuel and also that the fuel has been penetrated by jets of coolant.

The trigger mechanism is also well supported by experiments in which the existence of a threshold in coolant temperature has been established³. For constant coolant temperature below the threshold, the dwell time, defined as the time between initial contact of the two liquids and the start of the interaction,

and also the violence of the interaction, have been measured as functions of the initial fuel temperature. The initial shape of the dwell time curve, concave upwards, and indeed the very existence of a dwell time, supports the boiling curve explanation and the proposal of a critical heat flux. The violence of the interaction also increases with initial fuel temperature, as expected because more energy is available for the interaction.

Perhaps the most important results obtained are the existence of a threshold coolant temperature and the thresholds for external pressure which control both the efficiency of energy released per cycle and the trigger mechanism itself.

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D. J. BUCHANAN
T. A. DULLFORCE

UKAEA Research Group,
Culham Laboratory,
Abingdon, Berkshire

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- ¹ Yang, K., *Nature*, **243**, 222 (1973).
- ² Witte, L. C., Cox, J. E., and Bouvier, J. E., *J. Metals*, **22**, 39 (1970).
- ³ Brauer, F. E., Green, N. W., and Mesler, R. B., *Nucl. Sci. Engg.*, **31**, 551 (1968).
- ⁴ Groeneweld, P., *J. Heat Transfer*, **94**, 236 (1972).
- ⁵ Katz, D. L., *Chem. Engng. Prog.*, **68**, 68 (1972).
- ⁶ Katz, D. L., and Sliepcevich, C. M., *Hydrocarb. Proc. Petrol. Refin.*, 240 (November 1971).
- ⁷ Enger, T., Hartman, D. E., and Seymour, E. V., *Cryogenic Engineering Conf., NBS, Boulder, Colorado* (1972).
- ⁸ Döring, W., *Z. physik. Chem.*, **B**, **36**, 371 (1937).
- ⁹ Board, S. J., Farmer, C. L., and Poole, D. H., *Report, RD/B/N2423* (Central Electricity Generating Board, 1972).
- ¹⁰ Farber, E. A., and Scorah, R. L., *Trans. Am. Soc. Mech. Engrs.*, **70**, 369 (1948).
- ¹¹ Plesset, M. S., and Chapman, R. B., *J. Fluid Mech.*, **47**, 283 (1971).
- ¹² Buchanan, D. J., *J. Phys. D.* (in the press).
- ¹³ Christiansen, J. P., *Culham Laboratory Report, CLM-R106* (1970).
- ¹⁴ Batchelor, G. K., *Proc. R. Soc., A*, **213**, 349 (1952).

BIOLOGICAL SCIENCES

Induction of Hapten-specific B Cell Tolerance by Low Doses of Hapten-Carrier Conjugate

THE discovery that two cell types, thymus derived (T) and bursa or bone-marrow derived (B) cells, are involved in antibody production to some antigens was of particular importance to the study of immunological tolerance, as it became clear that inactivation of either cell line could result in unresponsiveness. Several workers have found that the two cell lines differ in the ease with which they may be made tolerant¹⁻³. Their findings support the general proposition that T cells are made tolerant more easily than B cells, in that they require a lower antigen dose and are made tolerant more rapidly. Thus tolerance induced by low antigen doses would only involve T cells, whereas higher doses could make both cell lines tolerant. As T cells recognize the "carrier" aspect of an antigen and B cells recognize and make antibody to the "haptenic" determinants, this concept explains the carrier-specificity of tolerance⁴, and the breakage of natural and acquired tolerance by cross-reacting antigens bearing "new" carrier determinants^{5,6}.

Nevertheless, there is some contradictory evidence to suggest that B cells can be made tolerant by low doses of antigen and under similar conditions to T cells^{7,8}. Support for this view is contained in this report, in which hapten-specificity is used as the criterion of B cell tolerance.

Using dinitrophenyl (DNP) as a hapten and rabbit γ globulin (RGG) as carrier, it has been found that hapten-specific tolerance could be induced in mice by a single low dose (30 μ g) of a conjugate carrying 5 to 6 DNP groups per RGG molecule, which has been centrifuged to remove particulate material. A similar conjugate bearing 1 DNP per RGG molecule produced only carrier-specific tolerance at low doses, and a much larger amount (500 μ g) had to be injected to produce hapten-specific tolerance. Thus, B cell tolerance is shown to be governed by either the degree of conjugation or the dose of the hapten-carrier complex. One implication is that, provided multiple binding of antigen to B cell can occur, B cells may be made tolerant by low antigen doses.

Hapten-protein conjugates were prepared by reaction of dinitrobenzenesulphonic acid (Eastman) with either rabbit γ globulin (RGG) Cohn fraction 11 or rabbit serum albumin (RSA) (both obtained from Koch Light) after the method of Eisen⁹. The conjugates were characterized as DNP_{0.8}-RGG, DNP_{5.7}-RGG, and DNP₄-RSA. The method used to induce tolerance was to centrifuge DNP_{0.8}-RGG and DNP_{5.7}-RGG at 30,000g for 60 min to remove particulate material¹⁰, and then to inject different amounts of the particle-free conjugates as a single intraperitoneal injection into Balb/c mice. Control groups received saline in place of tolerogen. Ten days later the mice were challenged with 30 μ g of either the same DNP-RGG conjugate used to induce tolerance, or of DNP₄-RSA. All challenge injections were given in Freund's complete adjuvant (FCA) intraperitoneally. Fourteen days later the mice were bled and their sera titrated for anti-DNP and anti-RGG activity, by measuring the extent of inactivation of phage T4 to either (DNP-T4) or RGG (RGG-T4). The "modified T4 phage" were prepared according to the methods of Carter *et al.*¹¹ and Haimovich and Sela¹². The titres given in Table 1 are the dilutions of serum which produced 50% inactivation of modified phage in 2 h, expressed as geometric means ($\pm$ s.d.) of ten sera per group.

The results of this experiment are shown in Table 1. Both DNP_{0.8}-RGG and DNP_{5.7}-RGG were shown to be good immunogens when administered in FCA, giving rise to good 14 d primary antibody responses to both hapten and

carrier. Pretreatment with 30 μ g centrifuged DNP_{0.8}-RGG led to almost complete tolerance to both DNP and RGG when challenge was given with the homologous conjugate DNP_{0.8}-RGG in adjuvant but this tolerance was not hapten specific, as challenge with DNP₄-RSA led to a normal anti-DNP response. In contrast, when a higher dose of DNP_{0.8}-RGG was used to induce tolerance (500 μ g), the mice did not produce anti-DNP to either DNP_{0.8}-RGG or DNP₄-RSA in FCA.

I therefore conclude that this very lightly coupled conjugate led only to carrier-specific tolerance at low doses, but gave both hapten and carrier tolerance at high doses. A different situation was seen when DNP_{5.7}-RGG was used as the tolerogen. Both the low dose (30 μ g) and the high dose (500 μ g) led to complete unresponsiveness which could be shown in both cases to be hapten-specific by challenge with DNP₄-RSA. Thus, it is clear that tolerance to a hapten can be achieved by low doses of conjugate, providing a suitable coupling ratio of hapten/carrier is used.

As an interpretation of these results, it may be suggested that B cells can be made tolerant by doses of antigen no higher than those required to make T cells tolerant, provided a sufficiently strong binding of antigen to the B cell can ensue. In this case, the difference between DNP_{0.8}-RGG and DNP_{5.7}-RGG as tolerogens is that each molecule of the former can only bind by a single bond, on average, to B cells, whereas the latter could be bound by up to six bonds per molecule, subject perhaps to limitation by steric factors. This would result in vastly stronger binding of the more heavily coupled conjugate to B cells. Karush¹³ has shown, for example, that the association constant for double binding of bivalent antibody to a hapten conjugate is of the order of 10,000 times higher than that for binding of hapten at a single combining site. It is very likely that this factor of multiple binding is decisive in leading to the low threshold for B cell tolerance with DNP_{5.7}-RGG. It is highly significant, in this respect, that two other examples in which B cell tolerance has apparently been readily obtained, relate to the determinants of the Fab portion of the γ globulin molecule, which could obviously bind simultaneously at two sites per antigen to B cells^{7,8}. Also of importance is the use of heterologous γ globulin as carrier for hapten in the induction of tolerance. RGG was chosen because it is an excellent tolerogen in itself, especially when prepared in particle-free form by high speed centrifugation. The use of a highly tolerogenic carrier doubtless helped to avoid immunization effects which could otherwise have masked low zone B cell tolerance, and is analogous to the use by other workers of non-immunogenic polypeptides¹⁴ or autologous proteins¹⁵ as carriers in achieving hapten tolerance.

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M. J. TAUSSIG

Immunology Division,
Department of Pathology,
Tennis Court Road, Cambridge

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- ¹ Taylor, R. B., *Transplant. Rev.*, **1**, 114 (1969).
- ² Weigle, W. O., Chiller, J. M., and Habicht, G. S., *Transplant. Rev.*, **8**, 3 (1972).
- ³ Mitchison, N. A., in *Cell Interactions and Receptor Antibodies in Immune Responses* (edit. by Mäkelä, O., Cross, A., and Kosunen, T. U.), 149 (Academic Press, London and New York, 1971).
- ⁴ Weigle, W. O., *J. Immun.*, **94**, 177 (1965).
- ⁵ Weigle, W. O., *J. exp. Med.*, **122**, 1049 (1965).
- ⁶ Benjamin, D. C., and Weigle, W. O., *J. exp. Med.*, **132**, 66 (1970).
- ⁷ Iverson, G. M., and Dresser, D. W., *Nature*, **227**, 274 (1970).
- ⁸ Taussig, M. J., *Eur. J. Immun.*, **1**, 367 (1971).
- ⁹ Eisen, H. N., in *Methods in Medical Research*, **10**, 94 (Year Book Medical Publishers, Chicago, 1964).
- ¹⁰ Dresser, D. W., *Immunology*, **5**, 378 (1962).
- ¹¹ Carter, B. G., Yo, S. L., and Schon, A. H., *Canad. J. Biochem.*, **46**, 261 (1968).

Table 1 Anti-DNP and Anti-RGG Titres in Sera of Balb/c Mice 14 d after Challenge with DNP-RGG or DNP-RSA Conjugates in FCA

Tolerogen	Dose (μ g)	Challenge Antigen	Mean antibody titres ($\pm$ s.d.)	
			Anti-DNP	Anti-RGG
—		DNP _{0.8} -RGG	1 : 4,320 ($\pm$ 104)	1 : 7,500 ($\pm$ 88)
		DNP _{5.7} -RGG	1 : 4,550 ($\pm$ 66)	1 : 8,660 ($\pm$ 110)
		DNP ₄ -RSA	1 : 5,200 ($\pm$ 84)	
DNP _{0.8} -RGG	30	DNP _{0.8} -RGG	1 : 280 ($\pm$ 24)	1 : 164 ($\pm$ 18)
		DNP ₄ -RSA	1 : 4,800 ($\pm$ 160)	
	500	DNP _{0.8} -RGG	1 : 64 ($\pm$ 18)	1 : 128 ($\pm$ 22)
		DNP ₄ -RSA	1 : 380 ($\pm$ 41)	
DNP _{5.7} -RGG	30	DNP _{5.7} -RGG	1 : 90 ($\pm$ 10)	1 : 110 ($\pm$ 12)
		DNP ₄ -RSA	1 : 116 ($\pm$ 40)	
	500	DNP _{5.7} -RGG	1 : 48 ($\pm$ 8)	1 : 140 ($\pm$ 21)
		DNP ₄ -RSA	1 : 32 ($\pm$ 8)	

Anti-DNP and anti-RGG titres were measured by inactivation of DNP-T4 phage or RGG-T4 phage (geometric means of $10 \pm$ s.d.). Mice were made tolerant by pretreatment with particle-free DNP-RGG conjugates in different doses, given in saline 10 d before challenge.

- ¹² Haimovich, J., and Sela, M., *Science, N.Y.*, **164**, 1279 (1969).
¹³ Karush, F., in *Specificity of Serological Reactions: Landsteiner Centennial, Ann. N.Y. Acad. Sci.*, **169**, 56 (1970).
¹⁴ Katz, D. H., Hamaoka, T., and Benacerraf, B., *J. expl. Med.*, **136**, 1404 (1972).
¹⁵ Golan, D. T., and Borel, Y., *J. expl. Med.*, **134**, 1046 (1971).

Quantitative Correlation between the Proton-carrying and Respiratory-stimulating Properties of Uncoupling Agents using Rat Liver Mitochondria

THE observation that dinitrophenol stimulates respiration and decreases work performance¹ by uncoupling oxidative phosphorylation² has stimulated great interest in the mechanism of action of uncoupling agents. The "chemical theory" of oxidative phosphorylation proposes that uncouplers catalyse the hydrolysis of a high energy intermediate of oxidative phosphorylation ($x \sim e$)^{3,4}, although the existence of such an intermediate remains speculative. Alternatively, Mitchell's "chemiosmotic hypothesis"⁵ proposes that uncoupling agents facilitate proton translocation across the mitochondrial inner membrane; thus disrupting a pH gradient and membrane potential, which he has proposed to be the "proton motive force" supporting ATP synthesis. The demonstration that uncouplers accelerate the decay of pH gradients following the addition of HCl to mitochondrial suspensions, provided initial support for this mechanism of action⁶. Subsequent reports have demonstrated a close relationship between alteration of proton conductivity of artificial bilayer membranes and uncoupler potency^{7,8}, although this conclusion has been disputed^{9,10}. Nevertheless, effects on artificial membranes may not be expected to correlate absolutely with an action on mitochondria. For this reason, we have compared the proton-transporting and respiratory-releasing properties of uncoupling agents, using intact mitochondria.

Isolated liver mitochondria were prepared from 150 to 250 g male Sprague-Dawley rats¹¹. All mitochondria had a respiratory control ratio¹² greater than 4.0 in a medium containing: 0.25 M sucrose, 15 mM KCl, 5 mM P_i , 5 mM $MgCl_2$, 1 mM EDTA, 10 mM triethanolamine Cl, pH 7.4, with 3.6 mM β -OH butyrate as substrate. Respiration was measured polarographically with a vibrating platinum electrode (Gilson Medical Electronics). Swelling was assayed by measuring changes in optical density at 520 nm with a Guilford 240 recording spectrophotometer.

The experimental basis for our approach of quantifying mitochondrial proton permeation^{13,14} is illustrated in Fig. 1 which depicts passive mitochondrial swelling in various isotonic solutions. A mechanistic explanation for these results is presented in the accompanying diagrams. Rapid mitochondrial swelling occurs in sodium, or ammonium (not shown) acetate. This is because sodium and ammonium enter the mitochondria by processes which diminish intramitochondrial H^+ (refs 13, 15, 16), while acetate transport results in proton entry. Charge and pH equilibrium are thus maintained during net sodium or ammonium acetate uptake. In the other case, where isotonic sodium or ammonium chloride are used, little swelling is noted. This is because chloride, the anion of a strong acid, must cross the mitochondrial membrane electrogenically as Cl^- . This creates an imbalance of charge and H^+ concentration, which prevents net salt penetration. The addition of dinitrophenol (DNP) induces rapid swelling in sodium or ammonium chloride, seen as the dotted lines. This does not appear to be a non-specific effect of DNP on mitochondrial permeability, since stimulation of swelling in isotonic sodium or ammonium acetate, potassium chloride, or sucrose are not similarly noted. We therefore conclude that in these conditions uncoupler-induced passive mitochondrial

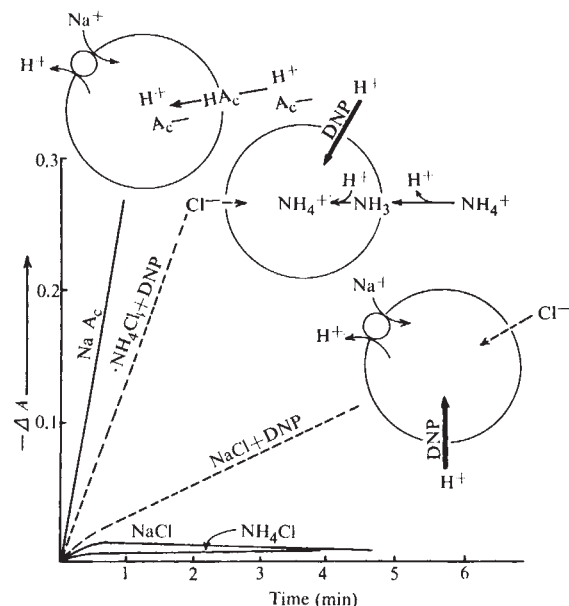


Fig. 1 Effects of DNP on passive swelling of rat liver mitochondria in various isotonic solutions. The final concentration of sodium or ammonium salts was 0.15 M. In all studies rotenone, 10^{-6} M; antimycin, 0.05 μ g, and triethanolamine Cl, 10 mM, pH 7.4, were present in a total volume of 2.5 ml. The temperature was 30°C. Changes in absorbance at 520 nm are recorded.

swelling is a valid, albeit indirect measurement of net proton transport.

The ability of uncoupling agents to facilitate passive swelling in NaCl was correlated with their respiration-stimulating properties over a wide range of uncoupler concentration. Measurements of swelling and respiration were simultaneously but separately performed in similar incubation conditions, except that appropriate respiratory inhibitors were present in the swelling studies. The data obtained with salicylanilide 13 (S-13) are shown in Fig. 2. No effect of S-13 on passive swelling in NaCl was noted, until the concentration reached 5×10^{-10} M. This was the same point at which respiratory stimulation in the sucrose medium was first noted. Ten other uncoupling agents were studied in identical fashion (Fig. 3). The minimal concentration of uncoupler needed to facilitate passive swelling in NaCl is plotted against the minimal concentration which stimulated respiration in NaCl or sucrose. An excellent correlation between these parameters is seen. In

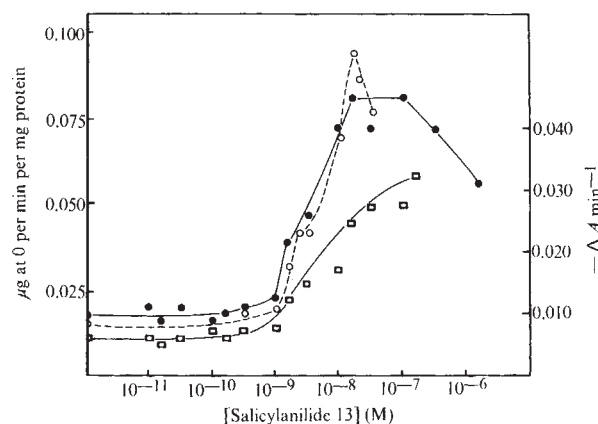


Fig. 2 Effects of salicylanilide 13 on passive swelling and respiration. Swelling studies ($\square$) were performed in 0.15 M NaCl in conditions identical to those described for Fig. 1, except that 3.6 mM β -OH butyrate was also present. Respiration was measured under identical conditions except that respiratory chain inhibitors were not added. 0.15 M NaCl ($\circ$) or 0.25 M sucrose ($\bullet$) were used with 10 mM TeacI, pH 7.4, and 3.6 mM β -OH butyrate as substrate.

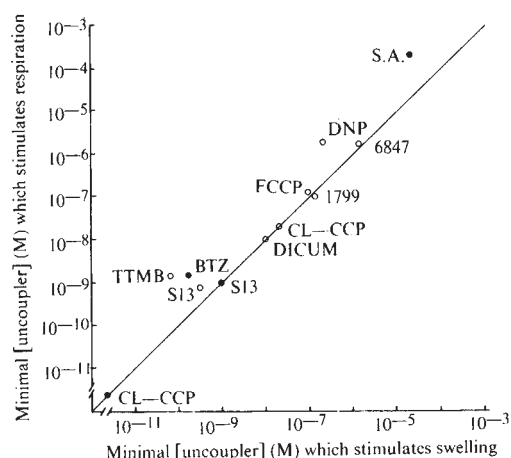


Fig. 3 Correlation between swelling and respiratory stimulation of various uncoupling agents. This graph summarizes a series of studies, with different uncoupling agents, in conditions identical to those described for Fig. 2. DNP, dinitrophenol; BTZ, SH-benzothiazole; S.A., thiosalicylic acid; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenyl hydrazone; CICC, carbonylcyanide-*m*-chlorophenyl hydrazone; 1799, 2,2'-bis(hexafluoroacetyl) acetone; Dicum, dicumarol; 6847, SF 6847; TTMB, 2-trifluoromethyl-tetrachloro benzimidazole; S13, salicylamide 13.

addition, those points not directly on the line fall somewhat to the left, indicating an effect on swelling at concentrations below that which stimulates respiration. Previous studies have indicated that the uncouplers SH-benzothiazole and thiosalicylic acid show poor correlation between effects on mitochondria and on artificial membranes⁹. This disparity is not apparent in our experiments.

It should be emphasized that the uncoupler-induced stimulation of passive swelling cannot be attributed to disruption of oxidative phosphorylation, since this process has been blocked by the presence of rotenone. The uncoupling therefore seems to be directly related to an alteration of membrane proton permeability. The extremely close correlation between the actions of uncouplers on the passive permeability properties of non-respiring non-phosphorylating mitochondria, and on respiratory stimulation strongly discounts direct catalysis of a high energy intermediate¹⁷.

These results do not rule out the possibility of a "proton pump" supported by $x \sim e^{18}$, nor do they support the fundamental propositions of Mitchell's chemiosmotic hypothesis. Nevertheless, the demonstration of a close quantitative correlation between the proton-transporting and respiratory-stimulating properties of uncoupling agents strongly supports Mitchell's original proposal that these agents uncouple oxidative phosphorylation by promoting electrogenic hydrogen ion transport across the mitochondrial inner membrane.

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JULIA CUNARRO
MICHAEL W. WEINER

Veterans Administration Hospital,
Department of Medicine and
Institute for Enzyme Research,
University of Wisconsin,
Madison, Wisconsin 53705

Received May 14, 1973.

- ¹ Lardy, H. A., and Phillips, P. H., *J. biol. Chem.*, **149**, 177 (1943).
- ² Loomis, W. F., and Lipmann, F., *J. biol. Chem.*, **173**, 807 (1948).
- ³ Slater, E. C., *Nature*, **172**, 975 (1953).
- ⁴ Lardy, H. A., and Wellman, H., *J. biol. Chem.*, **195**, 215 (1952).
- ⁵ Mitchell, P., *Nature*, **191**, 144 (1961).
- ⁶ Mitchell, P., and Moyle, J., *Biochem. J.*, **104**, 588 (1967).
- ⁷ Hopfer, U., Lehninger, A. L., and Thompson, T. E., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 484 (1968).

- ⁸ Liberman, E. A., Topaly, U. P., Tsofin, L. M., Jasaitis, A. A., and Skulachev, U. P., *Nature*, **222**, 1076 (1969).
- ⁹ Ting, H. P., Wilson, D. F., and Chance, B., *Arch. Biochem. Biophys.*, **141**, 141 (1970).
- ¹⁰ Wilson, D. F., Ting, H. P., and Koppelman, M., *Biochemistry*, **10**, 2897 (1971).
- ¹¹ Johnson, D., and Lardy, H. A., in *Methods in Enzymology*, **10**, 94 (1967).
- ¹² Chance, B., *Ciba Found. Symp. Regulation of Cell Metabolism*. (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 91 (Little Brown, Boston, 1959).
- ¹³ Brierey, G. P., et al., *J. biol. Chem.*, **245**, 5404 (1969).
- ¹⁴ Young, J. H., Blondin, G. A., and Green, D. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1364 (1971).
- ¹⁵ Mitchell, P., and Moyle, J., *Eur. J. Biochem.*, **9**, 149 (1965).
- ¹⁶ Chappell, J. B., and Crofts, A. R., in Tager, J. M., et al., *Regulation of Metabolic Processes in Mitochondria*, 293 (Elsevier, New York, 1966).
- ¹⁷ Wilson, D. F., *Biochemistry*, **8**, 2475 (1969).
- ¹⁸ Skulachev, V. P., *Current Topics in Bioenergetics*, **4**, 127 (1971).

Proton Magnetic Resonance and the Composition of Proteins

HERE we report experiments indicating that high resolution, high frequency proton magnetic resonance (PMR) could be used for determination of the amino acid combination of proteins.

PMR measurements on high molecular weight substances present serious difficulties because of the substantial width of the individual lines, broadened due to high correlation times of the slowly tumbling molecules^{1,2}. It is impossible to identify more than a small fraction of the protons present even in the low molecular weight (<20,000) proteins. But the chemical shifts of the protons of the twenty principal amino acids exhibit a fairly wide range of values of about 900 Hz at an operating frequency of 100 MHz (ref. 2).

We have attempted to observe PMR of acid hydrolysed protein to check whether such a spectrum resembles those obtained by mixtures of individual amino acids from the shelf. Fig. 1 shows such PMR spectra taken with a Varian XL-100 spectrometer at 100 MHz, and show that: the two spectra are almost identical; the individual lines have a line width of 1 to 2 Hz, as expected from individual amino acids; and the separation of the well defined lines is sufficient to identify their origin and to be able to estimate quantitatively their concentrations.

The protein, horse cytochrome *c* (purchased from Miles-Seravac Ltd) was hydrolysed for 24 h at 105° C in 6 N HCl, at a proportion of 1 ml HCl to 1 mg protein, used as purchased. After hydrolysis the sample was dried in a rotatory evaporator and redissolved in D₂O at a concentration of 50 mg of protein per ml, and at pD 2.

Sweeps of 500 s were used to obtain the spectra (Figs 1, 2), one for the region from 0 to 5 p.p.m. and one covering the region between 5 and 10 p.p.m., from the TSP internal standard. All the lines were identified through comparisons with spectra of individual amino acids.

Figure 2 represents the cytochrome sample of Fig. 1a, b after addition of histidine corresponding to 6 histidine residues per molecule. The relative amplitude of the signal is increased.

In Table 1 we give the known composition of horse cytochrome *c*, and a number which identifies the amino acids with the lines of spectra (Figs 1 and 2). The amplitude of the signal per residue, as observed in our experiment, shows (Table 1) that small changes in amino acid composition could be easily detected. We believe that the PMR method could be usefully applied to screen large numbers of samples in search of mutants. The method has the virtue of being much faster than chromatographic methods, and the preparation of the samples is identical in both methods. It is unsuitable where availability of the protein is limited, because samples must contain ~10–20 mg of protein (at 100 MHz).

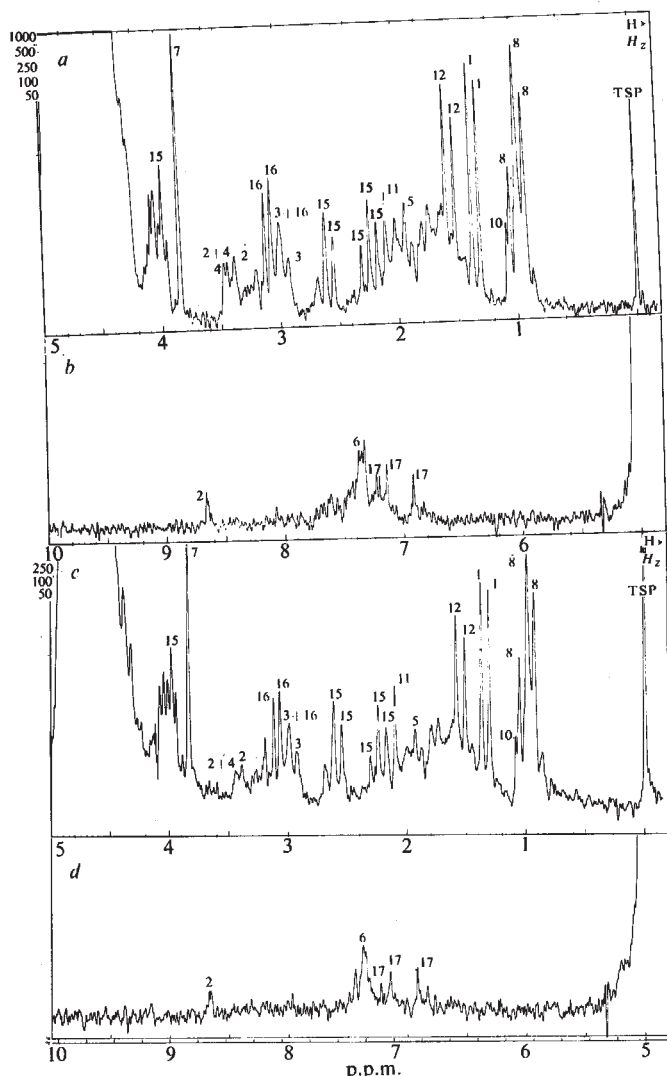


Fig. 1 *a* and *b*, 100 MHz PMR of cytochrome *c* (horse) 50 mg ml⁻¹, hydrolysed, pD 2, *T*=35° C. Each sweep 500 s. *c* and *d*, 100 MHz PMR of mixture of individual amino acids, hydrolysed, total concentration 50 mg ml⁻¹, pD 2, *T*=35° C. Each sweep 500 s.

But this is not an important problem for many applications, such as, for example, large scale population screening of easily obtainable proteins (such as haemoglobin).

Table 1 Assignments of Acids which Produce the Lines in Figs. 1 and 2

Amino acid	Abundance	Identification	mm per residue (approximate)
Thr	10	1	18
His	3	2	8
Arg	2	3	30
Pro	4	4	7
Lys	19	5	5
Phe	4	6	13
Gly	12	7	19
Ile + Leu	12	8	15
Val	3	10	7
Met	2	11	30
Ala	6	12	21
Glu + Gln	12	15	23
Asp + Asn	8	16	7
Tyr	4	17	6
Cys	2	—	—
Trp	1	—	—
Ser	—	—	—
Total	104		

Assignment of Arg, Pro, Lys is tentative. Cys and Trp destroyed during hydrolysis.

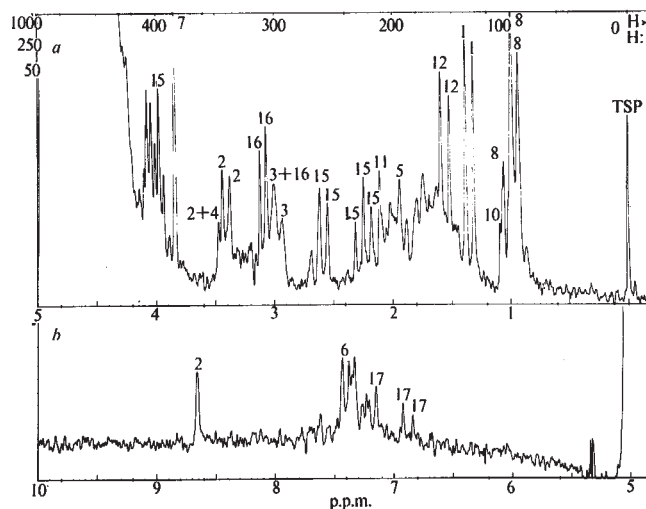


Fig. 2 *a* and *b*, 100 MHz PMR of cytochrome *c* (horse) of Fig. 1, after addition of equivalent of six histidine residues per molecule. Conditions as in Fig. 1.

The absolute numbers of residues could be obtained by comparing the amplitudes of the lines with a standard. Comparison of areas (integrations) are not very reliable because of the large number of lines. We have, however, not noticed any variations in line widths as long as the conditions of the experiments (pD, temperature) are identical.

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G. BEMSKI

*Instituto Venezolano de Investigaciones Científicas,
IVIC, Aptdo. 1827
Caracas, Venezuela*

T. HYNES*

*Instituto de Física, Pontificia Universidade Católica,
Rio de Janeiro, Brasil*

Received December 28, 1972.

* Present address: IIII Brandywine Blvd., Wilmington, Delaware 19809, USA.

¹ McDonald, C. C., and Phillips, W. D., *J. Amer. Chem. Soc.*, **91**, 1513 (1969).

² Jardetzky, O., and Wade-Jardetzky, N. G., *Ann. Rev. Biochem.*, **40**, 605 (1971).

Proton Radiographic Detection of Strokes

SOME penetrating radiations other than X rays can be used for the visualization of bodily structures. We are investigating the clinical potential of protein radiography, by which it is possible to obtain radiographs of unusually high contrast¹. Having visualized tumours within human specimens, including brain autopsy material², and carcinomas within breast tissue (manuscript in preparation), we now report the visualization of cerebral vascular lesions in specimens fixed in formalin.

Our method, described earlier², involves the external beam of 160 MeV protons from the Harvard Cyclotron. The beam, which is initially 0.5 cm in diameter, is made to strike a scatterer of lead or copper. Although this degrades the beam energy to about 137 MeV, at a distance of 3 m downstream it produces a large beam, uniform to a radius of 10 cm from the beam centre-line. This is used to illuminate the specimen. To show primarily tissue density variations, the effects of shape irregularities are minimized by immersing the specimen in water or other tissue-equivalent medium contained within a plastic box with parallel faces. Photographic film held against the exit face of this box is exposed

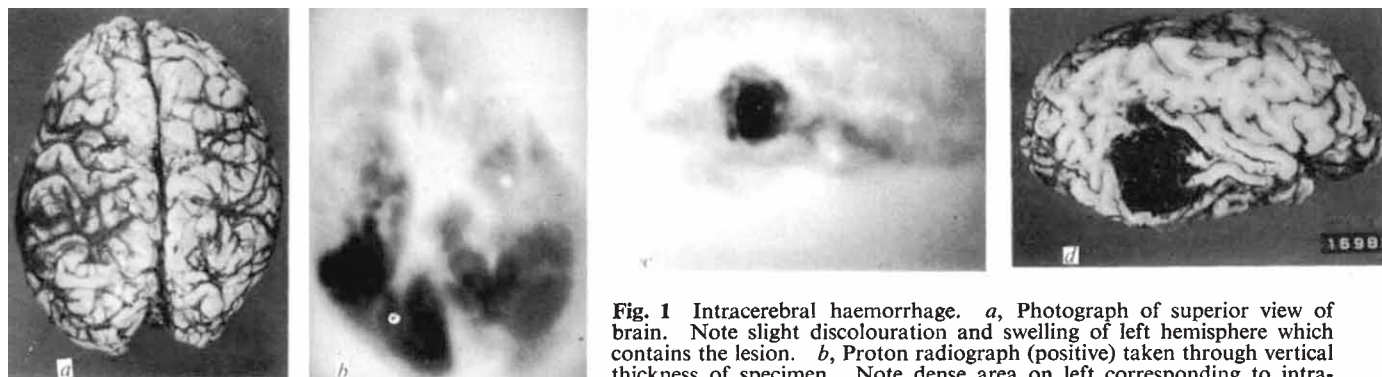


Fig. 1 Intracerebral haemorrhage. *a*, Photograph of superior view of brain. Note slight discoloration and swelling of left hemisphere which contains the lesion. *b*, Proton radiograph (positive) taken through vertical thickness of specimen. Note dense area on left corresponding to intracerebral blood clot. *c*, Proton radiograph (positive) of brain viewed transversely with specimen immersed in calcium chloride solution the density of which approximates that of the brain. (Specific gravity 1.038). *d*, Photograph of side view of brain showing blood clot.

to the emerging protons in the region of most rapid attenuation.

The eleven human brain autopsy specimens investigated included a case with a subdural haematoma, and one with a basilar artery aneurysm. Also included were six whole brains, of which three contained intracerebral haemorrhages, and three cerebral infarctions. Figure 1 demonstrates the typical findings on a whole brain specimen with an intracerebral blood clot. For comparison, Fig. 2 shows the visualization of a hemispheric infarction due to recent occlusion of the right middle cerebral artery.

Intracerebral blood clots were more opaque to protons than the surrounding brain tissue (Fig. 1), while regions of cerebral infarction were less dense than normal cerebral tissue (Fig. 2).

Optimal X radiographs were taken of these specimens, both in the immersion medium and in air, using a Machlett OEG

X ray tube with half-wave rectification. In addition, some specimens were X rayed at 2 MeV using a Van de Graaff electrostatic accelerator. The results of these X radiographic investigations corroborate our previous observation that under identical specimen-taking conditions the proton technique offers better visualization of tissue structures and of pathological changes within them². As no steps were taken to reduce the radiation dose to the specimens, a first surface dose of 125 rad was used for each exposure.

As far as we know, this is the first time that proton radiography has been used to demonstrate cerebral vascular lesions within human brain tissue. The only similar work on human tissues has been ours (ref. 2 and manuscript in preparation) including the measurement of the stopping power of protons in human skullbones³. Work on other animal tissues has included the production of proton images of a living rat⁴, and the use of multiple Coulomb scattering to demonstrate the internal structures of a mouse⁵. Contrast within thin tissue sections has also been demonstrated by microradiography employing alpha particles from ²¹⁰Po (ref. 6).

Work is in progress to develop proton radiographic techniques for the diagnosis of pathological states in the living human. Dose reduction has been achieved by the use of intensifying screens or a scintillator viewed by television⁷. As Fig. 3 shows, however, useful contrast is obtained over a limited range of total thickness, therefore immersion in a suitable fluid is required to neutralize surface irregularities. Where immersion is not feasible a proton beam image- pro-

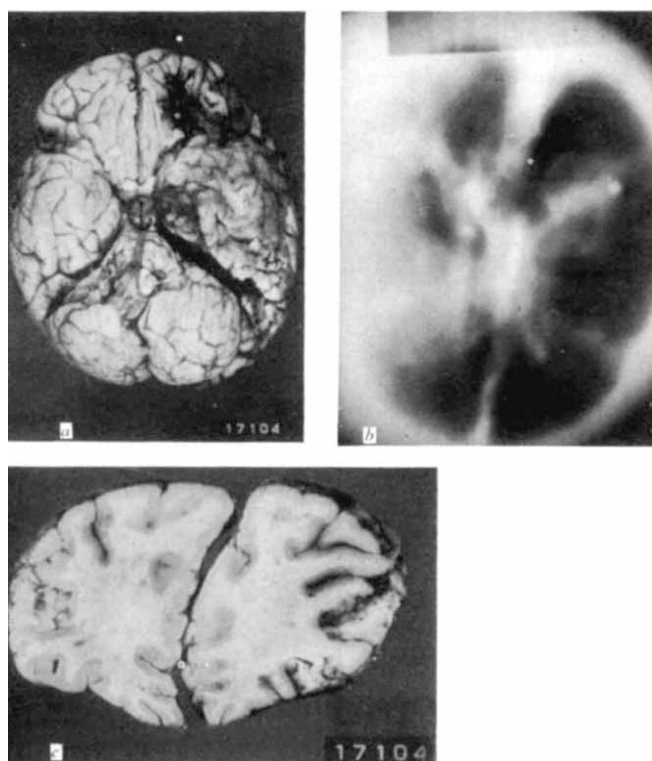


Fig. 2 Cerebral infarction. *a*, Photograph of inferior view of brain with massive infarction of left hemisphere (on right in picture) due to recent thrombotic occlusion of the left middle cerebral artery. *b*, Proton radiograph (positive) of brain. Note less dense left hemisphere lying inferiorly. *c*, Photograph of coronal sections through frontal lobes of brain with infarcted hemisphere on right.

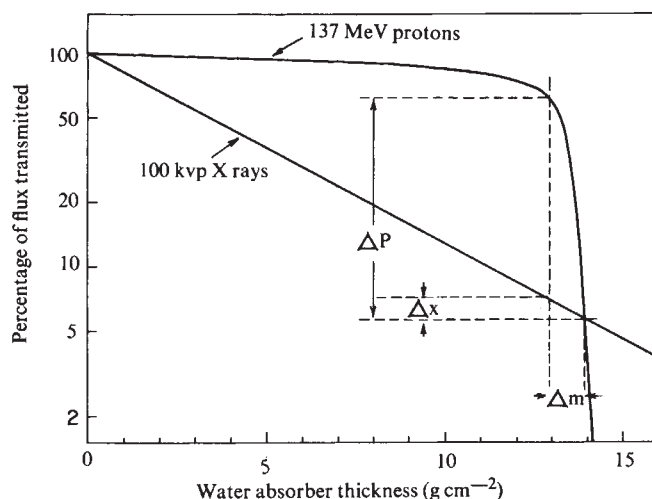


Fig. 3 Flux-depth curves for protons and X rays passing through a homogeneous medium. Note the difference in available contrast between the two rays for the detection of mass differences.

cessing system capable of reconstructing density anomalies within the patient appears to be necessary^{8,9}.

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V. W. STEWARD

Department of Pathology,
University of Chicago,
Chicago, Illinois 60637

A. M. KOEHLER

Cyclotron Laboratory,
Harvard University,
Cambridge, Massachusetts 02138

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- ¹ Koehler, A. M., *Science, N.Y.*, **160**, 303 (1968).
- ² Steward, V. W., and Koehler, A. M., *Science, N.Y.*, **179**, 913 (1973).
- ³ Koehler, A. M., Dickinson, J. G., and Preston, W. M., *Radiat. Res.*, **26**, 334 (1965).
- ⁴ Jung, B., *Report GWI-RZ/68* (Gustaf Werner Institute, Uppsala, Sweden).
- ⁵ West, D., and Sherwood, A. C., *Nature*, **239**, 157 (1972).
- ⁶ Belanger, L. F., and Belanger, C., *J. biophys. biochem. Cytol.*, **6**, 197 (1959).
- ⁷ Berger, H., Lapinski, N. P., and Beyer, N. S., *Proc. Eighth Symp. Nondestructive Evaluation in Aerospace, Weapons Systems and Nuclear Applications* (Southwest Research Institute, San Antonio, Texas, 1971).
- ⁸ Cormack, A. M., *Phys. Med. Biol.*, **18**, No. 2, 195 (1973).
- ⁹ Goitein, M., *Preprint LBL-547* (Lawrence Berkeley Laboratory, 1971).

Effect of Antitumour Polysaccharides on the Higher Structure of Serum Protein

MANY polysaccharides obtained from different origins have recently been found to inhibit the growth of transplantable solid tumours in mice¹⁻⁷.

Recently we have studied the structure and the antitumour activity of lentinan, pachyman and carboxymethylpachyman¹⁻⁴ and we consider that certain substances inherent in

the host may interact with the antitumour polysaccharides because the higher structure⁸ of the polysaccharides is associated with the appearance of activity and because there is an optimum dosage of the antitumour polysaccharides⁹. Clarification of the nature of these unknown substances, regardless of their character (humoral or cellular), may be important for studying the host defence mechanism against transplantable tumours.

We investigated the interaction of the antitumour polysaccharides with serum protein and found that the activity of the polysaccharides relates to the change of the higher structure of serum protein by the polysaccharides.

The polysaccharide was dissolved or suspended in 1/15 M phosphate buffer solution (pH 5.3), to a concentration of 10^{-2} M except for lentinan, and was then sterilized at 120° C for 20 min. All the polysaccharides then became completely soluble in the buffer solution. Crystallized bovine serum albumin (Miles Laboratories, Inc.) was dissolved in the same buffer (1 weight/volume %). Equal volumes of bovine serum albumin and the polysaccharide solution (4 ml) were mixed at room temperature for 20 min on a shaker. Then the optical rotatory dispersion was measured and the values a_0 and b_0 , the latter of which corresponds to the content of α -helix structure in serum albumin¹⁰, were calculated according to the Moffitt-Yang equation. The results obtained are shown in Table 1, which also shows the suppressing effect against sarcoma 180 (S180) in mice and the structure of the polysaccharides.

The larger the value of $|b_0|$, the greater the antitumour effect of the polysaccharides; all the polysaccharides which have a larger $|b_0|$ than that of the control experiment show antitumour action. None of the polysaccharides which have a smaller $|b_0|$ than that of the control experiment, however, had any suppressing effect against (S180).

There might well be a close relationship between antitumour activity and $|b_0|$, that is the content of α helix in the protein. We consider that most of the antitumour polysaccharides may affect the protein moiety (humoral or cellular) in the host at the first stage of the activity, which is followed by many biological-immunological changes.

The biological significance of the change of α -helix content of serum protein, is being studied. The allosteric effect of these polysaccharides on serum protein suggests relating the anti-

Table 1 Interaction of Polysaccharides with Bovine Serum Albumin

Polysaccharides	$-b_0$	Antitumour activity against S180 in mice		Structure
		Inhibition ratio (%)	Dose (mg kg ⁻¹) × 10	
Lentinan	278	100	1	$\beta(1\rightarrow3)$ Glucan
Hydroxyethylpachyman	278	100	5	$\beta(1\rightarrow3)$ Glucan
Hydroxypropylpachyman	270	99.3	5	$\beta(1\rightarrow3)$ Glucan
LC11	270	93.6	5	$\beta(1\rightarrow4)(1\rightarrow6)$ Glucan
Screloglucan	270	90.4	2.5	$\beta(1\rightarrow3)(1\rightarrow6)$ Glucan
GE3	258	91.3	100	$\beta(1\rightarrow6)$ Glucan
Carboxymethylpachyman	258	100	25	$\beta(1\rightarrow3)$ Glucan
Control	256	—	—	—
Starch	256	Not effective		$\alpha(1\rightarrow4)$ Glucan
Laminarin	248	1.5	25	$\beta(1\rightarrow3)$ Glucan
Carboxymethylcellulose	244	4.5	10	$\beta(1\rightarrow4)$ Glucan
Pachyman sulphate	244	12.3	25	$\beta(1\rightarrow3)$ Glucan
Hydroxyethylstarch	240	—10.0	10	$\alpha(1\rightarrow4)$ Glucan
Heparin	234	Not effective		Mucopolysaccharide
Pachyman sulphate	224	34.8	25	$\beta(1\rightarrow3))$ Glucan
Dextran	216	Not effective		$\alpha(1\rightarrow6)$ Glucan
Inulin	192	Not effective		Fructan

Optical rotatory dispersion was measured after mixing of 5×10^{-3} M polysaccharide and 0.5% bovine serum albumin solution in 1/15 M phosphate buffer (pH=5.3). For lentinan, the concentration was 4×10^{-3} M.

tumour activity and inflammation, because protein denaturation is said to induce a certain type of inflammation¹¹⁻¹⁵. Recent work shows that these antitumour polysaccharides inactivate the third component of complement and might have a certain relation to the results reported here¹⁶. Also the close relationship between activity and the α -helix content of serum protein may help to differentiate the active and inactive polysaccharides.

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JUNJI HAMURO

Central Research Laboratories,
Ajinomoto Co., Inc.,
1-1, Suzuki-cho, Kawasaki

GORO CHIHARA

National Cancer Centre,
Research Institute,
5-1, Tsukiji, Chuo-ku, Tokyo

Received June 4, 1973.

- ¹ Chihara, G., Hamuro, J., Maeda, Y. Y., Arai, Y., and Fukuoka, F., *Nature*, **225**, 943 (1970).
- ² Chihara, G., Maeda, Y. Y., Hamuro, J., Sasaki, T., and Fukuoka, F., *Nature*, **222**, 687 (1969).
- ³ Chihara, G., Hamuro, J., Maeda, Y. Y., Arai, Y., and Fukuoka, F., *Cancer Res.*, **30**, 2776 (1970).
- ⁴ Hamuro, J., Yamashita, Y., Ohsaka, Y., Maeda, Y. Y., and Chihara, G., *Nature*, **333**, 486 (1971).
- ⁵ Komatsu, N., Okubo, S., Kikumoto, S., Kimura, K., Saito, G., and Sakai, S., *Gann*, **60**, 137 (1969).
- ⁶ Nakahara, W., Fukuoka, F., Maeda, Y., and Aoki, K., *Gann*, **55**, 283 (1964).
- ⁷ Shibata, S., Nishikawa, Y., Tanaka, M., Fukuoka, F., and Nakanishi, M., *Z. Krebsforsch.*, **71**, 102 (1968).
- ⁸ Hamuro, J., Maeda, Y. Y., Arai, Y., Fukuoka, F., and Chihara, G., *Chem. Biol. Interaction*, **3**, 69 (1971).
- ⁹ Maeda, Y. Y., Hamuro, J., and Chihara, G., *Int. J. Cancer*, **8**, 41 (1971).
- ¹⁰ Moffit, W., Yang, J. T., *Proc. natn. Acad. Sci. U.S.A.*, **42**, 596 (1965).
- ¹¹ Mizushima, Y., and Suzuki, H., *Arch. int. Pharmacodyn.*, **157**, 115 (1965).
- ¹² Mizushima, Y., *Arch. int. Pharmacodyn.*, **149**, 1 (1964).
- ¹³ Mizushima, Y., *Lancet*, **i**, 169 (1965).
- ¹⁴ Opie, E. L., *J. exp. Med.*, **117**, 425 (1963).
- ¹⁵ Skidmore, I. F., and Whitehouse, M. W., *J. Pharm. Pharmacol.*, **17**, 671 (1965).
- ¹⁶ Okuda, T., Yoshioka, Y., Ikekawa, T., Chihara, G., and Nishioka, K., *Nature new Biol.*, **238**, 59 (1972).

Defects in Tactile Directional Sensitivity after Forebrain Commissurotomy in Man

SURGICAL transection of the cerebral commissures causes certain "deconnexion" or "split brain" symptoms in man¹ which manifest themselves as unilateral agnosias²⁻⁵, aphasia^{6,7} or dyspraxia⁸ depending on the method of examination. A major reason for the symptoms is that many types of behaviour depend on a nervous afferent input available from only half of the peripheral field to each hemisphere and the field halves fail to unite in the absence of the cerebral commissures²⁻⁵. Another reason is the "lateralization" of the cerebral hemispheres with regard to language^{6,7,9}, the perception of spatial relations¹⁰⁻¹³ and visual form¹⁴.

The "deconnexion" symptoms are most evident in tests involving activation of different types of peripheral receptors in a complex spatial and temporal order, for example visual or tactual recognition of objects, but can sometimes be observed also in tests using simple stimuli; for example inability to verbalize a plain colour presented in the left visual half-field¹. All tests involving simple stimuli, however, do not produce such symptoms; changes in skin temperature or the presence of touch

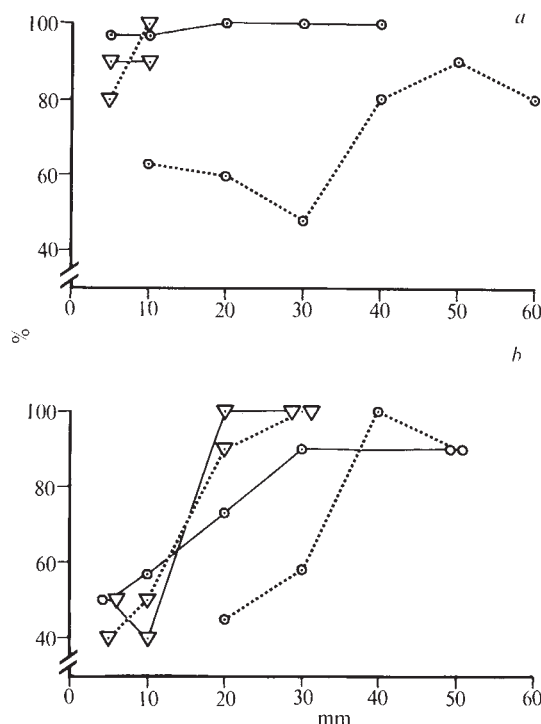


Fig. 1 Tactile directional sensitivity on the right (—) and left (---) cheeks of a 35-yr-old woman 5 yr after transection of the cerebral commissures. *a*, The subject's ability, in % correct responses, to indicate the direction of a stimulus moving continuously over the distance marked on the abscissa; *b*, the ability to indicate the directional relation between two discrete touch stimuli made sequentially at points separated by the distance marked on the abscissa. Each point represents 10 to 40 trials. O, Tests involving a verbal readout; ▽, tests involving a non-verbal readout.

stimuli are recognized verbally by the commissurotomy patients from either body half^{1,3,15}. The lack of symptoms in these instances is attributed to projection to each hemisphere of sufficient information by means of ipsilateral afferent pathways. It has also been suggested, on the basis of indirect physiological evidence, that for this reason "deconnexion" symptoms should not be expected at all in the face region with simple tactile stimuli.

Four right-handed patients, with the corpus callosum and the anterior and hippocampal commissures transected as a treatment for intractable epileptic seizures¹⁶, were examined for their ability to recognize and discriminate simple tactile stimuli (C.C., a 17 yr old schoolboy who was operated on at the age of 13; N.G., 35 yr old housewife operated on at 30; R.M., 29 yr old male operated on at 27; R.Y., 45 yr old male operated on at 43; for case histories see ref. 17). The previously reported lack of "deconnexion" symptoms for simple tactile stimuli was confirmed. The patients were thus able to report verbally the detailed location of a point touched on either half of the face, and usually also on the rest of the body, except for the distal parts of the limbs on the left side. They were unable to tell the direction of motion of a dowel drawn across the left palm, as has been reported previously¹, but when the test was extended to other parts of the body they were found to be functionally "split" down the midline contrary to expectations.

The test was performed in such a way that the rear, wooden part of a small camel-hair brush was drawn lightly across the skin surface in one of three directions. The patients who were blindfolded had been instructed to say "up" or "upwards" for a caudo-cranial movement on the head, neck or body, or a disto-proximal movement on the limbs. The expressions "down" or "downwards" were used for movements in the opposite direction and "sideways" or "across" for movements

at right angles to these. The distances covered by the stimuli were always equal in all directions, for each peripheral field. No attempt was made at the beginning to define the absolute distances covered by the stimuli, which thus were longer on wide surfaces like the back than on narrow surfaces like the ventral side of the forearm.

All four patients were found consistently to make errors when reporting in which direction the stimulus had moved on the left side of the body. The reports were erroneous for the left cheek and neck, as well as, for example, the left palm or tibia. When the stimuli were applied on the right side the three older patients, R. Y., N. G. and R. M., made no errors. C. C. made errors on the right side, but fewer than on the left. The patients were instructed to describe the direction of motion by non verbal means in control tests; the best way being by mimicking the stimulation with the index finger of the hand on the stimulated side. No defect was observed in these circumstances, and there was no difference between the body halves. The defect which appeared with a verbal readout was therefore not caused by a primary sensory disorder, but was presumably due to the unilateral aphasia caused by the commissurotomy *per se*.

Figure 1a illustrates the defect as it appeared in N. G. when stimuli of defined distances were used (two experimental sessions, 3 d interval). The stimuli were applied to the right or left cheeks and the distance was determined by drawing the dowel between the dots of a 5 mm square pattern marked on the skin. The figure shows clearly that the patient was able to give an accurate verbal report of the direction of a motion covering 5 mm or more on the right cheek (Fig. 1a) but not for the left cheek. The patient's accuracy seemed to improve with regard to the left cheek at distances above 40 mm. When a non verbal readout was used, the patient's responses were equally correct for both cheeks.

Figure 1b illustrates a second test, which was run in parallel. All criteria, parameters and conventions were the same as above except for the stimulus, of which the patient was required to give the direction, consisting of two discrete light touches with the camel-hair brush applied sequentially at points separated by the distances shown on the figure. A distance of 20 mm or more was necessary for the patient to do this accurately and she was equally precise on either cheek when tested with a non verbal readout. The greater distance compared with that necessary to tell the direction of continuous motion is comparable with the findings in normal persons. In verbal reports regarding the directional relation of the second stimulus to the first, the patient was more accurate on the right cheek than on the left for shorter distances.

The two different tests were often run together such that the patient was given five trials of continuous motion first and then five trials of sequential stimuli. It was noted that the patient made verbal comments when these switches were made on the right cheek whereas the change from one type of test to the other always passed unnoticed on the left cheek. The patient's accuracy in reporting verbally the relation of the second stimulus to the first improved on the left cheek at distances of 40 mm or more, which is the same range where the patient's accuracy in reporting continuous motion seems to improve. It is thus possible that the verbal report of the stimuli on the left side depended on the same afferent system in both tests; that is, a system to which motion has no special informative value.

That commissurotomy patients show a "split" for tactile directional sensitivity should be considered in connexion with the recently demonstrated, directionally sensitive, neural units of the cerebral somatosensory cortex^{18,19}. These units have not been found in the cortical layers which receive the primary input from the thalamus¹⁹ and could thus represent a truly cerebral event; presumably a prerequisite for the appearance of a "split" following commissurotomy. It may also be noted that whereas continuous bilateral peripheral fields are found for non directionally, sensitive units in the face area those displaying directional sensitivity appear to stop at the midline¹⁸.

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ULF NORRSELL*

Division of Biology,
California Institute of Technology,
Pasadena, California

Received May 10; revised June 19, 1973.

* Present address: Department of Physiology, University of Göteborg, Fack, S-400 33 Göteborg 33.

- ¹ Sperry, R. W., Gazzaniga, M. S., and Bogen, J. E., in *Handbook of Clinical Neurology* (edit. by Vinken, P. J., and Bruyn, G. W.), **4**, 273 (North-Holland, Amsterdam, 1969).
- ² Gazzaniga, M. S., Bogen, J. E., and Sperry, R. W., *Neuropsychologia*, **1**, 209 (1963).
- ³ Gazzaniga, M. S., Bogen, J. E., and Sperry, R. W., *Brain*, **88**, 221 (1965).
- ⁴ Gordon, H. W., and Sperry, R. W., *Neuropsychologia*, **7**, 111 (1969).
- ⁵ Norrsell, U., *Acta physiol. scand.*, **79**, 35A (1970).
- ⁶ Gazzaniga, M. S., and Sperry, R. W., *Brain*, **90**, 131 (1967).
- ⁷ Sperry, R. W., and Gazzaniga, M. S., in *Brain Mechanism Underlying Speech and Language* (edit. by Darley, F. L.), 108 (Grune and Stratton, New York, 1967).
- ⁸ Gazzaniga, M. S., Bogen, J. E., and Sperry, R. W., *Arch. Neurol.*, **16**, 606 (1967).
- ⁹ Levy, J., Nebes, R. D., and Sperry, R. W., *Cortex*, **7**, 49 (1971).
- ¹⁰ Bogen, J. E., and Gazzaniga, M. S., *J. Neurosurg.*, **23**, 394 (1965).
- ¹¹ Levy-Agresti, J., and Sperry, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 1151 (1968).
- ¹² Nebes, R. D., *Cortex*, **7**, 333 (1971).
- ¹³ Nebes, R. D., *Brain*, **95**, 633 (1972).
- ¹⁴ Levy, J., Trevarthen, C., and Sperry, R. W., *Brain*, **95**, 61 (1972).
- ¹⁵ Levy, J., and Sperry, R. W., *Cortex*, **6**, 349 (1970).
- ¹⁶ Bogen, J. E., Sperry, R. W., and Vogel, P. J., in *Basic Mechanisms of the Epilepsies* (edit. by Jasper, H. H., Ward, A. A., and Pope, A.), 439 (Little, Brown and Co., Boston, 1969).
- ¹⁷ Bogen, J. E., *Bull. Los Angeles neurol. Soc.*, **34**, 73, 135 (1969).
- ¹⁸ Schwartz, D. W. F., and Fredrickson, J. M., *Brain Res.*, **27**, 397 (1971).
- ¹⁹ Whitsel, B. L., Roppolo, J. R., and Werner, G., *J. Neurophysiol.*, **35**, 691 (1972).

Colour Opponent Neurones in the Human Visual System

If two gratings with the same sinusoidal luminance profile, one vertically orientated and one horizontally orientated, are imaged by means of two projectors on a white screen, the appearance continuously changes. First one grating is seen clearly, then the other; in other words, the gratings are seen to alternate or rival. There are periods when both gratings are seen together, but there are never periods when both disappear¹.

For alternation to be observed the two gratings need to have a difference in orientation of at least 15° (ref. 1). This orientation selectivity is comparable to that found in single neurone studies in the cortex of cat^{2,3} and monkey⁴ and psychophysical studies^{5,6}.

As well as being selective for orientation, neurones are also selective for colour⁷⁻⁹. The colour coding in some of the geniculate cells seems to take the form of a green excitatory centre and a red inhibitory surround or *vice versa*; another type has a blue excitatory centre with yellow inhibitory surround or *vice versa*^{7,8}. Campbell and Howell¹ observed that the monocular rivalry was seen more dramatically if the gratings were of different colours, rather than black and white or the same colour. Could the increased rivalry for gratings of different colours be due to the involvement of colour opponent neurones in addition to the orientation selective property of these neurones? If this is so, one would predict that combinations of green and red or blue and yellow gratings would give higher alternation rates than any other combination.

Different combinations of the following centroid wavelengths were used to colour the gratings; green 527 nm, red 641 nm, blue 475 nm, yellow 576 nm. Interference filters with a narrow bandwidth were used. The spatial frequency of both gratings was 1 cycle per degree, the screen size 8° in diameter.

Table 1 Colour Rivalry in Gratings of Opponent Colours

	1 Black/white	2 Blue/green	3 Yellow/red	4 Green/yellow	5 Blue/red	6 Green/red	7 Blue/yellow
F. W. C.	12.8±1.0	12.8±0.6	20.6±1.4	23.4±1.4	21.7±1.2	33.3±0.7	35.7±1.6
J. A.	6.8±0.7	18.4±0.9	21.0±0.4	19.1±0.4	20.9±0.6	24.1±0.7	22.4±0.6
J. P. J. R.	12.2±0.6	14.7±0.8	16.4±0.4	20.0±0.9	19.8±1.4	27.7±1.5	33.3±1.2
J. M.	4.8±0.4	13.2±0.9	20.7±1.0	19.8±0.8	22.9±0.8	23.4±1.1	27.6±1.3
Mean	9.1±2.0	14.8±1.3	19.7±1.1	20.6±1.0	21.3±0.7	27.1±2.3	29.8±3.0

Results are for four subjects: mean ± s.e.

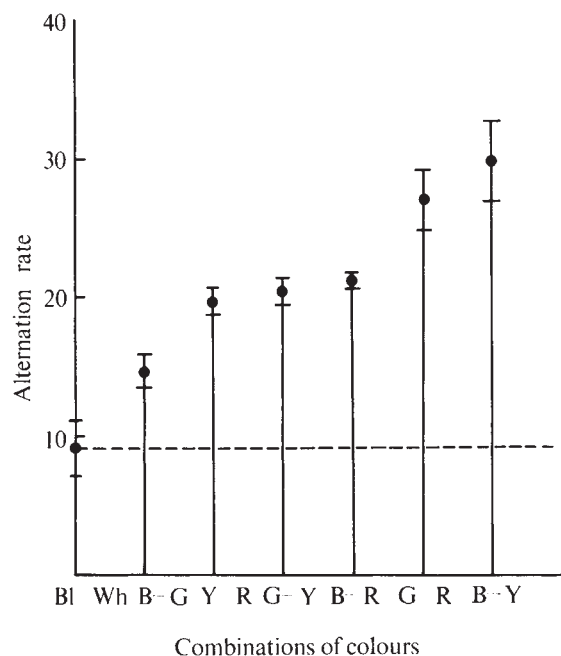


Fig. 1 The height of each line indicates the mean rate of alternation for each colour combination. The bars indicate ±1 s.e.

The luminance of the gratings did not seem to be critical provided that it was comfortably within the photopic range. For each colour combination the gratings were matched for apparent contrast; the contrast was approximately 0.3. The observer was asked to record each time there was a change from one grating dominating to the other. This rate of rivalry was measured for 1 min for each combination of colours. Nine 1-min runs were recorded for each combination in a quasi-random sequence and the mean and its s.e. calculated for each combination (Table 1). The pooled means of these results are shown at the foot of Table 1.

For crossed black and white gratings the mean rivalry rate was 9.1 changes per min (column 1). For all four subjects the rivalry rate for crossed green/red or blue/yellow gratings was much higher, giving means of 27.1 and 29.8 (columns 6 and 7). The rivalry rates for other colour combinations (columns 2 to 5) were intermediate between the black/white and green/red or blue/yellow results.

These results can be better assessed by inspecting Fig. 1. Here the pooled means ±1 s.e. are displayed. A dotted line is drawn through the mean rivalry rate for black/white gratings, below this line rivalry must be due only to the difference in orientation between the gratings. The additional rivalry caused by colour differences is shown by the height above the dotted line. The rates for green/red and blue/yellow are significantly greater than for any other combination.

Binocular rivalry thus depends not only upon the relative orientation but also the colour of the sine gratings. The differences in rivalry rates for different colour combinations cannot be due to accommodation or chromatic aberration for presbyopic and atropinized observers show the same magnitude of rivalry as observers with normal accommodation.

Nor can the difference be due to the absolute difference in

wavelength of the pairs of filters used, for if this were the case the blue/red combination (wavelength difference 166 nm [column 5]) should rival more than the blue/yellow combination (wavelength difference 101 nm [column 7]) and this is not so for any observer; in fact the reverse is found. Further, the difference in wavelength for the green/yellow combination is 49 nm, for the yellow/red combination 65 nm and for the blue/red combination 166 nm yet the rivalry rates for these three observations are not significantly different from one another (Fig. 1).

It seems likely therefore that monocular rivalry involves colour opponent mechanisms in human vision and the results are thus evidence for colour neurones in man which operate on a green-red, blue-yellow opponent system. Because the effect also depends on the orientation of the gratings these colour selective neurones must be as central, or more central, than the first orientationally selective neurones^{2,4}.

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JOSEF P. J. RAUSCHER
FERGUS W. CAMPBELL
JANETTE ATKINSON

*The Physiological Laboratory,
Cambridge CB2 3EG*

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- Campbell, F. W., and Howell, E. R., *J. Physiol. Lond.*, **225**, 19P (1972).
- Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **148**, 574 (1959).
- Campbell, F. W., Cleland, B. G., Cooper, G. F., and Enroth-Cugell, C., *J. Physiol. Lond.*, **198**, 237 (1968).
- Hubel, D. H., and Wiesel, T. N., *J. Physiol. Lond.*, **195**, 215 (1968).
- Campbell, F. W., and Kulikowski, J. J., *J. Physiol. Lond.*, **187**, 437 (1966).
- Blakemore, C., and Campbell, F. W., *J. Physiol. Lond.*, **203**, 237 (1969).
- De Valois, R. L., *J. gen. Physiol.*, **43**, 115 (1960).
- De Valois, R. L., *Cold Spring Harbor Symp. quant. Biol.*, **30**, 567 (1965).
- Gouras, P., *Science, N. Y.*, **168**, 489 (1970).

Spatial Frequency Selectivity of a Visual Tilt Illusion

ONE of the most consistent findings in neurophysiology^{1,2} and psychophysics³ has been that, in both human and animal visual systems, there exist neural mechanisms tuned to the orientation of a stimulus. It has been suggested that the selectivity of these orientation-sensitive detectors is sharpened by inhibitory interaction between them^{4,5}, and that illusions and distortions of apparent orientation occur as a consequence of that interaction⁶⁻⁸.

There are strong indications that "channels" selective for orientation are also selective for a fairly narrow band of spatial frequencies^{9,10}. I have now investigated the visual tilt illusion⁵ in a new format which provides the first evidence that, in man, orientation-specific inhibition operates between channels that have similar optimal spatial frequencies, and declines as the difference in frequency increases.

The stimulus display is shown in Fig. 1. It was presented against a dim background, for 0.5 s every 2 s during each

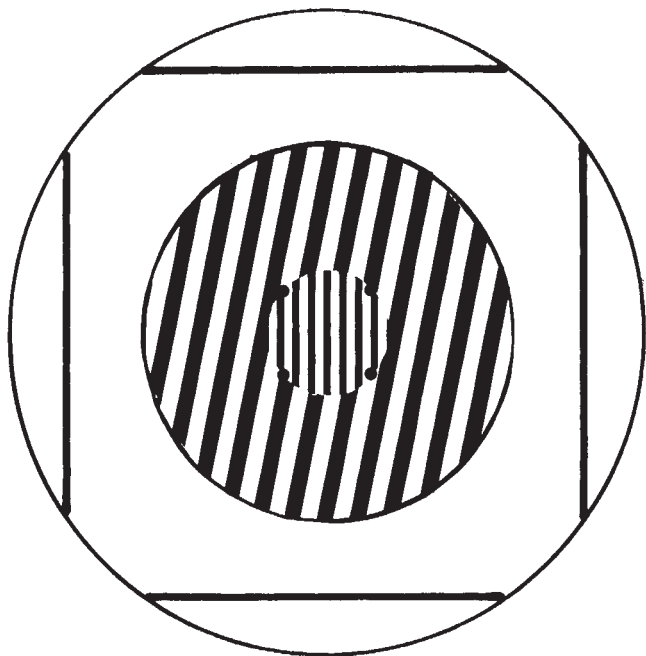


Fig. 1 The display as seen by the observer. The centre grating was 1.5 degrees in diameter with contrast=0.35, and the surrounding grating was 4.5 degrees in diameter, with contrast about 0.8. The dark horizontal and vertical lines surrounding the gratings provided the orientation reference. These reference lines were 5 min wide and their mid-points were 1 degree from the boundary of the outer grating. Four red spots, optically superimposed on the circumference of the centre grating, acted as a stimulus for accommodation when the display itself was off, but were not visible when it was on. All gratings in the experiment were sinusoidal photographs (though shown above, for convenience, as square waves). The mean luminance of each part of the display was 12 cd m^{-2} .

trial, to prevent the build-up of spatial-adaptation effects⁹. Each subject viewed the display with his left eye, without artificial pupil, and with his head supported by chin and temple rests. He was instructed to adjust the orientation of the gratings (whose relative angle was constant during a trial) until the centre grating appeared to be parallel to the fixed vertical reference lines (see Fig. 1). Use of a "subjective vertical" criterion was discouraged, to rule out Gibsonian "normalization" effects¹¹, but eye movements were allowed. Each setting of the motor-driven display took 15–30 s. Before each trial the display was offset by 10–15 degrees, alternately clockwise and anti-clockwise from the vertical.

For each run of five trials, the spatial frequency of the surround (2.5, 5, or 10 c per degree) and the angle between the gratings (+10, 0 or -10 degrees) were held constant, while the spatial frequency of the centre grating was varied in ascending or descending steps. The angle between the gratings was changed after each run, and the surrounding spatial frequency was changed after every three runs. The order of the different conditions was randomized within and between sessions.

Result were obtained from two male students who did not know the purpose of the experiment. In the control condition (0 degrees between the gratings) neither subject showed any significant response bias as a function of the spatial frequency of centre or surround gratings. Only for subject J. W. was there a small anti-clockwise bias of all control settings, with a mean shift of 0.6 degrees from true vertical. With ± 10 degrees between the gratings, however, the subjects' settings indicated that, as expected, the apparent orientation of the test grating was biased away from that of the surround. The extent of this induced-tilt effect is shown in Fig. 2 (a and b). The illusion has been plotted as half the difference between mean settings made in the +10, and in the -10 degree conditions. This procedure eliminated the small

baseline errors mentioned above, and also smoothed out any left-right asymmetries in the data.

For both 5 and 10 c per degree surrounds the tilt effect was maximum when the spatial frequencies of the test and surround gratings were equal. For the 2.5 c per degree surround, however, the curve peaked at a test frequency of 5 c per degree. An extra condition for subject H. G., with 1.25 c per degree surround, confirmed this low-frequency anomaly. Analysis of variance on the data of Fig. 2 (excluding the 1.25 c per degree condition) showed no significant difference between the subjects, but significant effects of test frequency, and of surround frequency (both with $P < 0.01$), and a significant interaction of test frequency with surround frequency ($P < 0.05$).

These results are very similar to those found for spatial-frequency specific after-effects⁹. In the case of after-effects with adapting frequencies greater than 3–4 c per degree, threshold elevation and the spatial-frequency shift are tuned to the adapting frequency, but with lower adapting frequencies the effects are tuned around 3–4 c per degree, with progressively lower amplitude. Similarly, in this experiment, results with 2.5 and 1.25 c per degree surrounds appear to be attenuated versions of the 5 c per degree curve. This is illustrated in Fig. 2c, where data averaged over the two subjects have been replotted as percentages of the peak value for each curve, against a normalized spatial-frequency axis, on which the test frequency is expressed in octaves, relative to the test frequency at which the maximum occurred. Also shown are data confirming the frequency-specific tilt effect with 5 and 10 c per degree surrounds, averaged over three other subjects for whom centre and surround contrasts were reduced by a factor of four, and display was continuous during a trial which took about 5 s. The results, therefore, did not depend upon having high contrast or flashing stimuli.

It thus seems that spatial-frequency selective channels^{9,12} are operative in this simultaneously induced tilt illusion. If a channel with a particular optimal orientation is inhibited

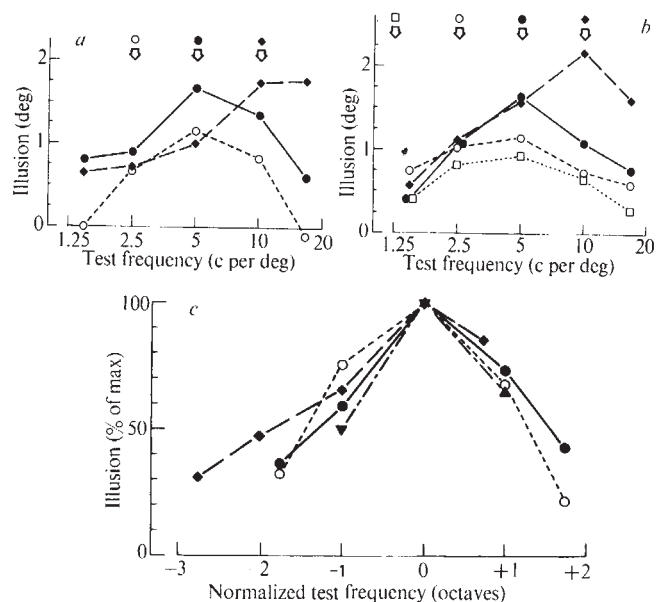


Fig. 2 a, Amount of illusion plotted as a function of test spatial frequency. Symbols denote surround frequency: 2.5 c per degree ($\circ$), 5 c per degree ($\bullet$), 10 c per degree ($\blacklozenge$). Arrows indicate where the peak of each curve would lie if the illusion were spatial frequency specific for all values of surround frequency. Each point was based on sixteen trials. Subject: J. W. b, As for a, but including data with a surrounding grating of 1.25 c per degree ($\square$). Subject: H. G. c, Data from a and b averaged, and re-scaled so that each curve is superimposed at 100% (see text). The illusion has very similar tuning characteristics for all surround frequencies. Also shown are similar results from three other subjects, as described in the text: 5 c per degree surround ($\blacktriangle$), 10 c per degree surround ($\blacktriangledown$). Other symbols as in a.

only by channels with the same or nearby orientations, then tilt contrast is predicted when spatially adjacent stimuli differ in orientation by a suitable amount⁵. The spatial-frequency specific tilt effect obtained here implies that such orientation-selective inhibition operates maximally between channels with similar frequency selectivities, and hardly at all between those tuned two octaves apart.

Blakemore and Tobin¹³ recently found complex units which were inhibited by gratings at or near the preferred orientation, placed outside the conventional receptive field. Bishop *et al.*¹¹ have shown that simple cells are inhibited by lines tilted away from the preferred orientation, even when the line is strictly confined to the discharge centre of the receptive field. The function of such feature-specific inhibition¹⁵ may be to enhance, or even produce¹⁶, the selectivity of visual neurones along important stimulus dimensions. In systems of neurones possessing a degree of wired-in specificity, the development of inhibition between neighbouring neurones would be an economical method of sharpening the selectivity of the units, provided that the dimensions for which the units were selective was topologically mapped in the cortex¹⁷. Just such a precise spatial organization of orientation-selective cells exists in the monkey's striate cortex. Oblique penetrations of the microelectrode revealed ordered arrays of units, with the axis of each successive receptive field rotated by about 10 degrees from that of the previous unit¹.

There is thus strong physiological support now for the inhibitory explanation of tilt illusions⁵. My results raise the possibility that spatial-frequency channels are similarly organized in the human brain such that physically adjacent channels respond best to neighbouring frequencies, and that their frequency selectivity is enhanced by mutual inhibition. Such a system could overcome the problem of having rather coarse spatial tuning of units at earlier levels in the visual pathway^{18,19}.

In view of recent reports that the tilt after-effect does not depend on spatial frequency^{20,21}, the finding of a simultaneous tilt illusion selective for spatial frequency supports the proposal²² that there is an inhibitory process in the spatial frequency domain which is different from the mechanism of adaptation to prolonged stimulation.

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MARK A. GEORGESON

Laboratory of Experimental Psychology,
University of Sussex,
Brighton BN1 9QY

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- ¹ Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **195**, 215 (1968).
- ² Henry, G. H., and Bishop, P. O., *Contributions to Sensory Physiology*, **5**, 1 (1971).
- ³ Campbell, F. W., and Kulikowski, J. J., *J. Physiol.*, **187**, 437 (1966).
- ⁴ Andrews, D. P., *Vision Res.*, **7**, 975 (1967).
- ⁵ Blakemore, C., Carpenter, R. H. S., and Georgeson, M. A., *Nature*, **228**, 37 (1970).
- ⁶ Wallace, G. K., *Percept. Psychophys.*, **5**, 261 (1969).
- ⁷ Bouma, H., and Andriessen, J. J., *Vision Res.*, **10**, 333 (1970).
- ⁸ Lennie, P., *Nature new Biol.*, **233**, 155 (1971).
- ⁹ Blakemore, C., and Campbell, F. W., *J. Physiol.*, **203**, 237 (1969).
- ¹⁰ Blakemore, C., and Nachmias, J., *J. Physiol.*, **213**, 157 (1971).
- ¹¹ Gibson, J. J., *J. exp. Psychol.*, **20**, 553 (1937).
- ¹² Campbell, F. W., and Maffei, L., *J. Physiol.*, **207**, 635 (1970).
- ¹³ Blakemore, C., and Tobin, E. A., *Exp. Brain Res.*, **15**, 439 (1972).
- ¹⁴ Bishop, P. O., Dreher, B., and Henry, G. H., *J. Physiol.*, **218**, 53P (1971).
- ¹⁵ Richards, W., *Vision Res.*, **12**, 1113 (1972).
- ¹⁶ Benevento, L. A., Creutzfeldt, O. D., and Kuhnt, U., *Nature new Biol.*, **238**, 124 (1972).
- ¹⁷ Blakemore, C., Iversen, S. D., and Zangwill, O. L., *Ann. Rev. Psychol.*, **23**, 413 (1972).
- ¹⁸ Sachs, M. B., Nachmias, J., and Robson, J. G., *J. opt. Soc. Am.*, **61**, 1176 (1971).
- ¹⁹ Maffei, L., and Fiorentini, A., *Vision Res.*, **13**, 1255 (1973).
- ²⁰ Campbell, F. W., and Maffei, L., *Vision Res.*, **11**, 833 (1971).
- ²¹ Parker, D. M., *Q. Jl exp. Psychol.*, **24**, 1 (1972).
- ²² Tolhurst, D. J., *J. Physiol. Lond.*, **226**, 231 (1972).

Steroid Hormone Production by Pig Blastocysts

A LONG-STANDING problem in reproductive biology is that of the maternal recognition of pregnancy, the mechanism by which the developing conceptus signals its presence to the mother. An essential requirement for the establishment and maintenance of pregnancy is that the normal ovarian cycle should be arrested and the activity of the corpus luteum prolonged. In the sheep and pig, the recognition signal has been transmitted even before the embryonic tissue becomes intimately attached to the uterine epithelium and is therefore clearly distinct from implantation. In species such as the pig the blastocyst, while it remains free in the uterine lumen, may produce some substance that is capable of diffusing into uterine "milk" and across the lumen, exerting a local effect on the uterine tissues.

Huff and Eik-Nes¹ showed that rabbit blastocysts contain enzyme systems capable of synthesizing cholesterol and pregnenolone from acetate and of metabolizing various steroid substances. Other workers^{2,3} have found a significant concentration of progesterone and smaller amounts of 20 α -dihydroprogesterone and 17 α -hydroxyprogesterone in the blastocyst and blastocoel fluid. Seamark and Lutwak-Mann³ suggested that the progestagens in blastocysts "are not necessarily synthesized by the embryos, but may be conveyed to them by the endometrial secretion". Work in this laboratory has demonstrated the presence of unconjugated oestrogens as well as progesterone in pig blastocysts, and so we decided to investigate the possibility that steroid hormones are produced by the blastocyst of this species. Such local steroid production may well have an essential role in the maternal recognition of pregnancy or in the process of implantation.

Some time before attachment to the endometrium, the blastocysts of the pig elongate enormously and occupy a large proportion of the length of the uterine horn. This elongation⁴ coincides closely with the time of the recognition of pregnancy⁵. About 3 d later, tenuous attachment occurs at isolated points along the length of the blastocyst, but the definitive attachment by interlocking microvilli is not seen until about 18 d after copulation (p.c.)⁶. Blastocysts were recovered by flushing the uterine horns with a large volume of sterile medium. The concentration of total unconjugated oestrogens⁷ and progesterone^{8,9} was determined by radioimmunoassay. The antiserum used for the estimation of total unconjugated oestrogens (SLC 6X) had a high cross-reactivity with oestradiol-17 β and oestrone¹⁰, whereas that used for progesterone (S49 No. 6) reacted predominantly with progesterone, 17 α -hydroxyprogesterone and 11-deoxycortisol⁹. Steroids were extracted from the blastocyst tissue with diethyl ether in the oestrogen assay and petroleum ether in the progesterone assay and they were not subjected to a separation procedure before radioimmunoassay.

Uterine flushings were centrifuged (2,000 r.p.m. for 5 min) and the weight of the residue, consisting predominantly of fragmented blastocysts, was taken as the original wet weight of the blastocysts. The concentration of total unconjugated oestrogens at days 14 and 17 was 2.23 and 0.86 ng g⁻¹ wet tissue, respectively, and that of progesterone at days 14 and 16 was 6.8 and 9.8 ng g⁻¹ wet tissue. During this period of pregnancy, the peripheral plasma concentration of progesterone is about 10 to 20 ng ml⁻¹ whereas that of total unconjugated oestrogens is less than 0.1 ng ml⁻¹.

To determine whether the blastocyst was actively synthesizing steroids or accumulating steroids derived from the mother, blastocyst tissue was incubated with appropriate precursors. Blastocysts (0.2 to 0.5 g wet weight), endometrial tissue (0.2 g) and uterine washings (3.0 ml) were incubated separately under sterile conditions in either a total volume of 4 ml of Medium 199 or Brinster's medium¹¹ for 3 h at 37° C and gassed with 95% oxygen:5% CO₂. The incubation medium was prepared with a recently purified ³H-labelled

Table 1 The % Incorporation of ^3H -Androstenedione and ^3H -Dehydroepiandrosterone into Phenolic Compounds, Oestrone and Oestradiol-17 β by Pig Blastocysts

Stage of pregnancy (d p.c.)	% Incorporation of initial radioactivity (% g ⁻¹ h ⁻¹)	
	Oestrone	Oestradiol-17 β
	Androstenedione	
10	Undetectable	Undetectable
14	13.6	2.4
15	6.9	1.4
16	23.2 (37.7) *	1.3 (2.2) *
	Dehydroepiandrosterone	
14	34.1	4.2
15	13.5	4.0
16	32.8 (39.4) *	2.2 (1.9) *

Each figure is the mean of duplicate determinations.

Substrates (about 1.0 μCi) incubated with blastocyst tissue in Medium 199.

* Results obtained using Brinster's medium¹¹ are given in brackets.

substrate (1,2- ^3H -androstenedione, 45.9 Ci mmol⁻¹; 7 α -dehydroepiandrosterone (DHA), 15.9 Ci mmol⁻¹; 1,2- ^3H -progesterone, 33.5 Ci mmol⁻¹; 7 α - ^3H -pregnenolone, 18 Ci mmol⁻¹; 6,7- ^3H -oestrone, 45 Ci mmol⁻¹; 6,7- ^3H -oestrone sulphate, 470 mCi mol⁻¹; or 1,2,6,7- ^3H -cortisol, 107 Ci mmol⁻¹) to provide an activity of approximately 1 μCi in each flask. After incubation, the flasks were rapidly frozen in liquid nitrogen and stored at -15° C until the reaction products were determined. Extraction, solvent partition, repeated thin-layer chromatography and derivative formation for the isolation, identification and determination of labelled oestrone and oestradiol-17 β and of progesterone were performed as previously described^{12,13}.

Table 1 shows the incorporation of labelled androstenedione and DHA expressed as the % incorporation of the substrate h⁻¹ g⁻¹ wet weight of tissue at different times after mating. On day 10, there was no significant incorporation of androstenedione into oestrogens, but by days 14, 15 and 16 the conversion of labelled DHA or androstenedione to oestrone and oestradiol-17 β was appreciable, the incorporation of DHA being about twice that of androstenedione. The overall ratio of oestrone to oestradiol-17 β was about 7:1 for both precursors. The sum of the radioactivity found in these two compounds represented 70 to 80% of the total radioactivity in the phenolic fraction. Because there was little difference between the two incubation media (Medium 199 and Brinster's medium) with respect to the % incorporation of androstenedione and DHA into oestrone and oestradiol-17 β (and of pregnenolone to progesterone), experiments were subsequently carried out with Medium 199 only.

Table 2 The % Incorporation and Metabolism of Various Substrates into Phenolic Compounds (Oestrone, Oestradiol-17 β) and Progesterone by Pig Blastocysts

Substrate	% Incorporation of initial radioactivity (% g ⁻¹ h ⁻¹)			
	Oestrone	Oestradiol-17 β	Progesterone	Unknown
Progesterone				
14 d p.c.	0.9	0.3	—	—
15 d p.c.	2.2	0.4	—	—
Cortisol				
15 d p.c.	<0.01	<0.01	—	—
Oestrone sulphate				
14 d p.c.	81.7	16.1	—	—
Oestrone				
14 d p.c.	33.3	24.7	—	—
Pregnenolone				
16 d p.c.	Not detected	Not detected	<5.0	19.1

Each figure is the mean of duplicate determinations.

Table 2 shows corresponding measurements using various substrates. The incorporation of labelled progesterone indicates that there was some conversion of this substrate to oestrone and oestradiol-17 β after 14 and 15 days of pregnancy. There was no detectable incorporation of labelled cortisol or labelled pregnenolone into oestrogens. The rapid hydrolysis of oestrone sulphate by the blastocyst tissue was striking in that all the radioactivity was recovered in a phenolic fraction largely in the form of labelled oestrone. Oestradiol-17 β was present in approximately the same ratio as was found in the previous experiments described in Table 1. Incubations with labelled oestrone confirmed the presence of a 17 β -oxidoreductase enzyme system within blastocyst tissue in that an appreciable proportion of radioactivity was incorporated into oestradiol-17 β . Table 2 also shows that the incorporation of labelled pregnenolone into progesterone was of a low order, although a significant proportion was incorporated into unidentified compounds more polar than progesterone in their chromatographic behaviour.

Table 3 Radiochemical Purity of Labelled Oestrone and Oestradiol-17 β Isolated from Blastocyst Tissue after Incubation with Various ^3H -labelled Substrates

Substrate	Product	Ratio of $^3\text{H} : ^{14}\text{C}$ after thin-layer chromatography		
		1st chromatogram	2nd chromatogram	3rd chromatogram
Androstenedione	Oestrone	119	111	117
		153	165	171
	Oestradiol-17 β	20	23	21
DHA		38	36	32
	Oestrone	319	340	316
		205	210	215
Progesterone	Oestrone	124	129	119
		30	39	32
	Oestradiol-17 β	38	43	43
		42	42	42
		4	13	6
		7	12	9

$^3\text{H} : ^{14}\text{C}$ ratios of oestrogens after extraction, solvent partition, repeated thin-layer chromatography and derivative formation.

After the addition of ^{14}C -oestrone and ^{14}C -oestradiol-17 β (~1,500 d.p.m. per 100 μg), blastocyst tissue was extracted with diethyl ether, a phenolic fraction was prepared by solvent partition, and oestrone and oestradiol-17 β separated by thin-layer chromatography (ethyl acetate:cyclohexane, 1:1). After acetylation, the oestrogen-3-monoacetates were chromatographed again (ethyl acetate:cyclohexane, 3:7), then hydrolysed, and the free oestrogens chromatographed a third time (ethyl acetate:cyclohexane, 1:1)^{10,13}.

The initial substrate concentration of labelled oestrone sulphate was much greater than that of the other substrates used as a result of its relatively low specific activity. The rapid hydrolysis of labelled oestrone sulphate is equivalent to the formation of at least 400 ng g⁻¹ h⁻¹ of oestrone, some 200 times the maximum endogenous concentration of immunoreactive unconjugated oestrogens in blastocyst tissue. There was little evidence for a sulphotransferase enzyme system concerned with the reverse reaction as less than 1% of oestrone was incorporated into oestrone sulphate or oestradiol-17 β sulphate. The incorporation of labelled androstenedione, DHA and progesterone into sulphated compounds was similarly low. There was, on the other hand, a substantial incorporation into other conjugated compounds which accounted for approximately 20% of the initial activity. Evidence for the radiochemical purity of labelled oestrone and oestradiol-17 β isolated after incubations of blastocysts with radioactive substrates is given in Table 3.

Labelled androstenedione, DHA or pregnenolone were also incubated with endometrial mince and uterine washing. There was negligible incorporation of these substrates into oestrone, oestradiol-17 β or progesterone in these incubations.

These results provide biochemical evidence for the presence

within the blastocyst tissue of aromatase, 17-20 desmolase and 3-sulphatase enzyme systems concerned with the production of oestrogens from neutral steroids, progesterone and conjugated steroids, respectively. Evidence has also been obtained for the presence of other enzyme systems involved in the formation of steroid conjugates other than the sulphates of oestrone and oestradiol-17 β . These findings indicate that the presence of unconjugated oestrogen and progesterone in blastocyst tissue is probably a result of their synthesis *in situ* and not a result of their diffusion from the maternal circulation. Thus, even at this early stage of development, the pig blastocyst has the enzymatic capacity to produce biologically important steroid hormones. It remains to be seen whether the local effect of hormones produced by the blastocyst is, in some way, concerned with the ongoing process of implantation or with the signal whereby the developing embryo conveys its presence to the mother. This latter event may be related to an alteration in the pattern of uterine metabolism.

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J. S. PERRY
R. B. HEAP
E. C. AMOROSO

ARC Institute of Animal Physiology,
Babraham, Cambridge

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- ¹ Huff, R. L., and Eik-Nes, K. B., *J. Reprod. Fert.*, **11**, 57 (1966).
- ² Lutwak-Mann, C., in *The Biology of the Blastocyst* (edit. by Blandau, R. J.), 252 (The University of Chicago Press, 1971).
- ³ Seamark, R. F., and Lutwak-Mann, C., *J. Reprod. Fert.*, **29**, 147 (1972).
- ⁴ Perry, J. S., and Rowlands, I. W., *J. Reprod. Fert.*, **4**, 175 (1962).
- ⁵ Dhindsa, D. S., and Dziuk, P. J., *J. Anim. Sci.*, **27**, 122 (1968).
- ⁶ Crombie, P. R., thesis, University of Cambridge, 1972.
- ⁷ Challis, J. R. G., Heap, R. B., and Illingworth, D. V., *J. Endocr.*, **51**, 333 (1971).
- ⁸ Heap, R. B., Gwyn, M., Laing, J. A., and Walters, D. E., *J. agric. Sci., Camb.*, **81**, 151.
- ⁹ Abraham, G. E., Swerdloff, R., Tulchinsky, D., and Odell, W. D., *J. clin. Endocr. Metab.*, **32**, 619 (1971).
- ¹⁰ Challis, J. R. G., Harrison, F. A., and Heap, R. B., *J. Endocr.*, **57**, 97 (1973).
- ¹¹ Brinster, R. L., *J. Reprod. Fert.*, **10**, 227 (1965).
- ¹² Bedford, C. A., Harrison, F. A., and Heap, R. B., *J. Endocr.*, **55**, 105 (1973).
- ¹³ Challis, J. R. G., Harrison, F. A., and Heap, R. B., *J. Endocr.* (in the press).

Electrical Coupling across Developmental Boundaries in Insect Epidermis

THE polarity of the surface folds on the abdominal cuticle of the insect *Rhodnius*, which normally run at right angles to the antero-posterior axis, is controlled by the underlying epidermal cells. When portions of integument are transplanted or rotated the epidermal cells produce surface folds the orientation of which shows that the cells "remember", at least to some extent, their original polarity^{1,2}. Such experiments also suggest that the cells have access to information which defines the spatial pattern of differentiation within each segment³. It has been suggested that a gradient of some property exists which is reiterated from segment to segment so that the intersegmental border intervenes between the "high" part of one gradient and the "low" of the next. In one model the segmental gradient is viewed as a concentration gradient of a diffusible morphogen²⁻⁵. Studies in a closely related insect have shown that from a very early stage the segments have independent lineages, clones stopping abruptly at the intersegmental border^{5,6}.

It has often been suggested that molecules conveying the information necessary for defining the spatial pattern of

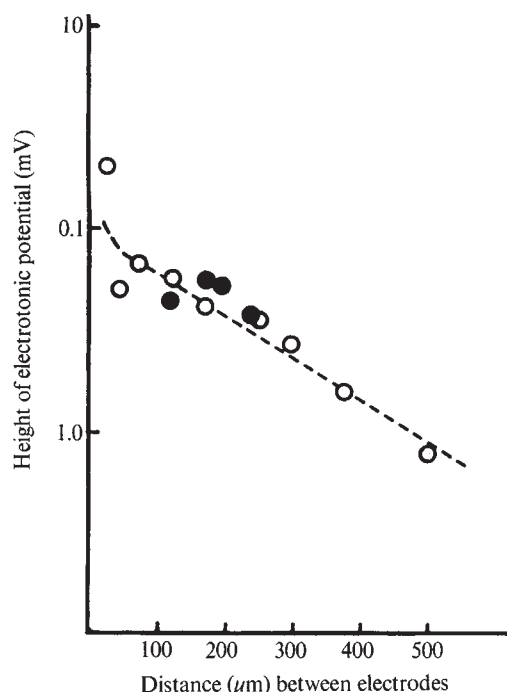


Fig. 1 Spread of ionic current in the insect epidermis. Ordinate: Height of the electrotonic potential (mV). Abscissa: Distance between current passing and voltage recording electrodes (μm). $\circ$, Measurements with both electrodes in the same segment; $\bullet$, electrodes in adjacent segments. Line drawn through the points calculated according to

$$V = \frac{IR_i}{4d} j H_0^{(1)}(j r/\lambda)$$

where V is the height of the electrotonic potential, I the injected current, d the thickness of the sheet, r the interelectrode distance and λ the space constant of the preparation (given by $\sqrt{R_m/R_i d}$ where R_m and R_i are the specific resistances of the surface membrane and intercellular pathway respectively). λ set at $350 \mu\text{m}$; $H_0^{(1)}$ is a tabulated Bessel function¹³.

differentiation may pass from cell to cell via low electrical resistance pathways⁷⁻⁹. There is little evidence to support these ideas, although Dixon and Cronly-Dillon¹⁰ have reported that gap junctions disappear from the central retina of *Xenopus* at the time of determination of retinal polarity. (The presence of gap junctions in cultured cells has been correlated with the ability to exchange small molecules such as nucleotides, and with low resistance intercellular pathways¹¹.) Any morphogen controlling differentiation in the insect segment could pass from cell to cell via low resistance junctions, although the independent behaviour of each segment requires that the movement of the morphogen be restricted to cells within the same segment. We have therefore examined whether low resistance intercellular pathways exist within each segment and from one segment to the next.

Third stage larvae of *Rhodnius* were used 3-5 d after feeding. The dorsal integument of the abdomen was removed (approximately $5 \times 3 \text{ mm}$), and the fat body and basement membrane stripped off. Examination in the electron microscope showed a monolayer of epidermal cells (about $10 \mu\text{m}$ in diameter) continuous across the intersegmental border. This preparation was pinned out flat in Jones and Cunningham medium¹² epidermal cells uppermost and viewed in a Zeiss dissecting microscope ($\times 100$). Glass microelectrodes filled with 3 M KCl or 0.8 M K citrate (tip potential $< -8 \text{ mV}$; resistances 40-60 M Ω) were used. Current pulses were injected into one cell and the voltage deflexion produced in a nearby cell recorded by a second intracellular microelectrode. Membrane potentials were routinely recorded on insertion of each electrode and one electrode was then switched over to current injection to ensure that both current passing and voltage recording electrodes lay inside a cell.

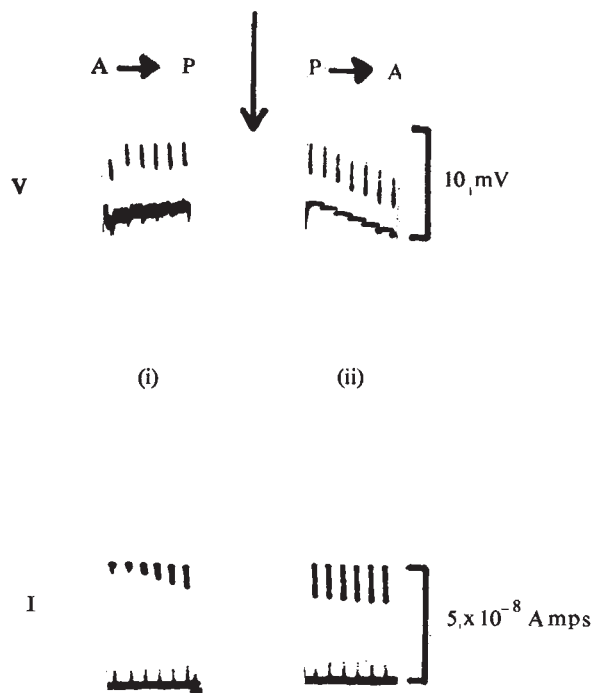


Fig. 2 Electrical coupling across the intersegmental border. In each case the upper record gives the resting membrane potential (lower margin) and the final height of the electrotonic potential (upper margin) at the end of a one second current pulse recorded on a slowly moving pen recorder. The lower record gives the magnitude of the injected current pulse. Depolarizing current pulses were passed. (i) Electrotonic potential recorded with current passing electrode in anterior part of one segment and voltage recording electrode across intersegmental border. (ii) Current passing switched onto the voltage recording electrode and *vice versa*, so current now injected in posterior part of one segment and recorded in anterior part of the next. Distance between electrodes = 125 μm .

Intracellular membrane potentials ranged from -6 to -30 mV with a mean of -17 mV (± 5.0 mV s.e.m.; $n=61$). Input resistances (measured with an electrode separation of <25 μm) were in the region of $10^6 \Omega$; on no occasion were the current passing and voltage recording electrodes in the same cell. After determination of the input resistance of the preparation the inter-electrode distance was increased gradually and the height of the electrotonic potential measured.

Preparations were tested for the spread of ionic current both within the same segment and across the intersegmental border. In all preparations ($n=13$) electrical coupling has been observed both between cells in the same segment and between cells in adjacent segments. A semi-logarithmic plot of the height of the electrotonic potential against the inter-electrode distance for one of these preparations is shown in Fig. 1. For this series of measurements the current passing electrode was placed in a cell in the posterior part of one segment and current spread measured away from this electrode in both anterior and posterior directions. The measurements made within the same segment and those made with the intersegmental border interposed between the electrodes fall along the same line, suggesting that the border does not present a significant barrier to the movement of ions from one segment to the next. The line drawn through the points in Fig. 1 was calculated using a solution of the cable equations for current spread from a point polarizing electrode into a flat sheet¹⁴ taking a space constant of 350 μm . When injection of current was switched from one electrode to the other so that current passed first in the antero-posterior direction and then in the reverse direction across the barrier (Fig. 2) the size of the electrotonic potential did not change; there was no evidence for rectification.

The cells of the insect epidermis secrete a waxy epicuticle which is highly impermeable to both water and ions^{15,16}. The

possibility that a shunt for the flow of ionic current exists in the extracellular space between the cells and this high resistance cuticle must not be overlooked. If such a shunt existed it could give rise to apparent electrical coupling between the cells because of ion current flow from one cell to the next via a restricted extracellular pathway.

Wigglesworth¹⁶ has shown that superficial abrasion renders the cuticle permeable to water. We have found that normal membrane potentials and electrical coupling within and between segments persist after heavy surface abrasion, which allows neutral red to penetrate the cuticle and enter the cells, showing that they are not damaged by this treatment. The input resistances of these epidermal cells were not markedly different from those in unabraded preparations. The intercellular coupling illustrated in Fig. 1 is thus the result of low resistance connexions between the cells and is not the consequence of a restricted extracellular pathway. There is, therefore, effective electrical coupling, both between cells situated in the same segment, and between cells in adjacent segments; suggesting that there is no simple correlation between the independent development of adjacent organs and the presence of an intervening barrier to the movement of small ions.

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ANNE E. WARNER

Department of Physiology,
Royal Free Hospital School of Medicine,
8 Hunter Street, London WC1

P. A. LAWRENCE

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH

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- ¹ Locke, M., *J. exp. Biol.*, **36**, 459 (1959).
- ² Lawrence, P. A., Crick, F. H. C., and Munro, M., *J. Cell. Sci.*, **11**, 815 (1972).
- ³ Lawrence, P. A., *Symp. Soc. exp. Biol.*, **25**, 379 (1971).
- ⁴ Lawrence, P. A., *J. exp. Biol.*, **44**, 607 (1966).
- ⁵ Lawrence, P. A., *Nature new Biol.*, **242**, 31 (1973).
- ⁶ Lawrence, P. A., *J. Embryol. exp. Morph.* (in the press).
- ⁷ Furshpan, E. J., and Potter, D. D., *Curr. Top. dev. Biol.*, **3**, 98 (1968).
- ⁸ Goodwin, B. C., and Cohen, M. H., *J. theor. Biol.*, **25**, 49 (1969).
- ⁹ Loewenstein, W. R., *27th Symp. Soc. dev. Biol.* (edit. by Locke, M.) (Academic Press, New York, 1969).
- ¹⁰ Dixon, J. S., and Cronly-Dillon, J. R., *J. Embryol. exp. Morph.*, **28**, 659 (1972).
- ¹¹ Gilula, N. B., Reeves, O. R., and Steinbach, A., *Nature*, **235**, 262 (1972).
- ¹² Jones, B. M., and Cunningham, I., *Exp. Cell Res.*, **23**, 386 (1961).
- ¹³ Jahnke, E., and Emde, F., *Tables of Functions with formulae and curves*, 3rd ed. (Tuebner, Leipzig, Berlin, 1938).
- ¹⁴ Noble, D., *Biophys. J.*, **2**, 381 (1962).
- ¹⁵ Wigglesworth, V. B., *Quart. Jl microsc. Sci.*, **76**, 270 (1933).
- ¹⁶ Wigglesworth, V. B., *J. exp. Biol.*, **21**, 97 (1945).

Optimal Fish Cruising Speed

THE life cycles of various species of fish include long range migrations for feeding and spawning purposes. Long stretches of these migratory routes are often crossed without food intake so that efficient use of the store of internal energy is essential to survival. Many such migratory species have produced various adaptations for efficient swimming such as streamlining and carangiform¹ body movements. Schooling, which is also common among long distance swimming species², has been shown to contribute to the locomotory performance³. Here I show that regulating the swimming speed can increase the performance and efficiency of motion by appreciable amounts.

Such theoretical predictions of optimal cruising speeds can be of aid in tracking and locating fish during migrations for fishing purposes and so on. Fishes have two propulsive mus-

cular systems⁴, the aerobic system for slow, protracted swimming (cruising) and the white anaerobic muscles chiefly for short bursts at high speeds. When considering movements over large distances, only speeds within the range for sustained swimming need to be taken into account. What, then, is the maximum range for swimming at constant speed with the total available energy constrained and given?

Defining W_p as the propulsive rate of work and W_m as the rate of energy expenditure on internal metabolic processes independent of swimming speed (standard rate⁵),

$$E = (W_p + W_m)t \quad (1)$$

where E is the total available energy and t the swimming time. The thrust T is equal in magnitude to the hydrodynamic drag for swimming at constant speed. At normal swimming velocities (Reynolds number much greater than 1) the drag is approximately proportional to the velocity u squared. Thus

$$T = \tau u^2 \quad (2)$$

where τ is a constant including the fish surface area, water density and a non-dimensional drag coefficient. The thrust can be related to the propulsive rate of work by

$$\eta W_p = Tu \quad (3)$$

where equation (3) defines the mechanical efficiency η of the fish propulsion mechanism. Experimental data for the dependence of η on u in steady swimming was recently obtained for rainbow trout⁶ for the range of sustained swimming speeds. From these results

$$\eta = \beta u \quad (4)$$

where β is an empirically obtained constant. Strictly speaking this relation is valid only for one species. Other related swimming characteristics, such as the dependence of oxygen intake^{6,7} and oscillation frequency^{8,9} on swimming speed, have, however, been found to hold for many species using carangiform and subcarangiform locomotion. Therefore it will be assumed that equation (4) also is of a more general nature. Substituting equations (2) and (4) into equation (3)

$$W_p = \tau u^2 / \beta \quad (5)$$

Equation (1) can now be written

$$E = ((\tau u^2 / \beta) + W_m)t \quad (6)$$

The distance crossed during this time when swimming in a straight horizontal line is

$$l = ut \quad (7)$$

Eliminating t from equations (6) and (7)

$$l = Eu / (\tau u^2 / \beta + W_m) \quad (8)$$

It is evident from equation (8) that l has a maximum for some positive value of u as $l=0$ for both $u=0$ and when u becomes very large. Differentiation of l with respect to the velocity yields

$$u^2_{\max} = \beta W_m / \tau \quad (9)$$

which is equivalent to (see equation (5))

$$(W_p)_{\max} = W_m \quad (10)$$

The maximum range possible with given total energy store is obtained when the swimming velocity is such that the rate of energy expenditure required for propulsion is equal to the standard (resting) metabolic rate.

The working rates W_p and W_m can be established from the relation between oxygen intake and swimming speed, which has been measured for various species of freshwater and marine fish⁶⁻⁷ including rainbow trout. The velocity for which condition (10) is fulfilled is approximately 1 fish length s^{-1} , which is well within the range of cruising speeds⁶ for which the aerobic system is used exclusively.

Having established the existence and approximate value of the optimum cruising speed as defined above, it remains to find the magnitude of the gains possible by optimizing the swimming speed.

Any speed can be written in terms of the optimal cruising speed $u_{\max}$ from the definition

$$u = \alpha u_{\max} \quad (12)$$

Substituting in equation (8)

$$l = \alpha u_{\max} E / ((\tau \alpha^2 u_{\max}^2 / \beta) + W_m) \quad (13)$$

and $l_{\max}$ is obtained by setting $\alpha=1$. Applying equation (9)

$$l/l_{\max} = 2\alpha / (1 + \alpha^2) \quad (14)$$

Figure 1 shows that the possible range is within 20% of the maximum for swimming speeds of one half to twice the optimal speed. Long range cruising speeds as inferred from tank tests are usually up to four lengths s^{-1} (ref. 10). For this speed, the range is less than half the maximum (taking $u_{\max} \approx 1$ length s^{-1}) showing that optimizing the cruising speed can have a marked influence on the possible range of movement.

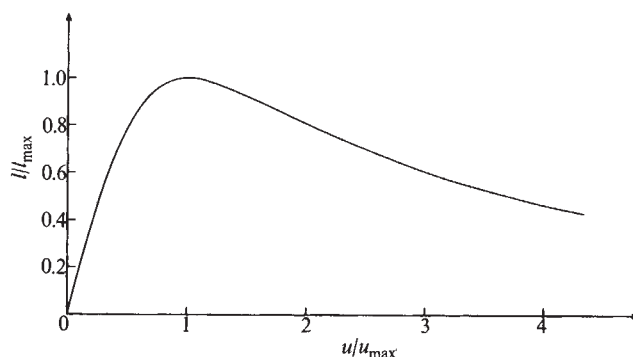


Fig. 1 Ratio of range to maximum range as a function of the normalized velocity for constant speed swimming.

A related problem is that of minimizing the energy required to cross a given distance. Applying equations (1) to (7) gave exactly the same result as the former problem, namely equation (9). This was to be expected as one problem is the inverse of the other.

The optimal cruising speed found above is rather low when compared with the maximum endurance speeds mentioned. Such endurance tests are, however, generally made in flowing water installations, so that the fish is not free to choose its preferred swimming speed. Some indication of actual cruising speeds may be obtained from data on continuously swimming species such as *Euthynnus affinis*, where cruising at about 1.3 to 1.7 lengths s^{-1} was observed^{12,18} with the lower limit approached after long food deprivation¹⁸.

On the other hand, the actual optimum speed for any given species or individual may be different from that defined in equation (10) because of the general model used. Thus, Webb¹⁴ found that the thrust-speed relation in trout is slightly different from equation (2), that is $T = \tau u^{1.79}$. Using this instead of equation (2) gives, in place of equation (10), $0.79(W_p)_{\max} = W_m$, which increases the optimum velocity by approximately 10%. Similar variations in the dependence of efficiency on speed have been observed (for goldfish¹⁵). As a result of such minor variations the value of the optimum velocity found (equation (10)) gives a rough idea of the optimum cruising speed. More accurate estimates for specific species or groups of fish can be obtained, given the exact dependence of thrust and efficiency on swimming speed.

Differences in these relationships between species may have evolved as a result of the differing requirements, such as river

migrations in which the swimming speed has to be higher, due to the faster counterflow, than in oceanic species.

D. WEIHS

Department of Applied Mathematics and
Theoretical Physics,
University of Cambridge,
Cambridge

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¹ Breder, C. M., jun., *Zoologica*, **4**, 159 (1926).

² Breder, C. M., jun., *Zoologica*, **52**, 25 (1967).

³ Weihs, D., *Nature*, **241**, 290 (1973).

⁴ Alexander, R., McN., *Functional Design in Fishes* (Hutchinson, London, 1967).

⁵ Brett, J. R., *J. Fish. Res. Bd. Can.*, **21**, 1183 (1964).

⁶ Webb, P. W., *J. exp. Biol.*, **55**, 521 (1971).

⁷ Tytler, P., *Nature*, **221**, 274 (1969).

⁸ Bainbridge, R., *J. exp. Biol.*, **35**, 109 (1958).

⁹ Hunter, J. R., and Zweifel, J. R., *Fish. Bull.*, **69**, 253 (1971).

¹⁰ Blaxter, J. H. S., *FAO Fish. Rep.*, **62**, 69 (1969).

¹¹ Hunter, J. R., *Fish. Bull.*, **69**, 267 (1971).

¹² Cahn, P. H., *Fish. Bull.*, **70**, 197 (1972).

¹³ Magnuson, J. J., *Copeia*, No. 1, 56 (1970).

¹⁴ Webb, P. W., *J. exp. Biol.*, **55**, 489 (1971).

¹⁵ Smit, H., Amelink-Koutsaal, J. M., Vijverberg, J., and von Vaupel-Klein, J. C., *Comp. Biochem. Physiol.*, **39A**, 1 (1971).

Evolutionary Implications of Collar Cell Ectoderm in a Coral Planula

THERE is only one account on the ultrastructure of cnidarian planula larvae (largely of the surface coat) of the hydrozoan *Corydendrium parasiticum*¹. In view of the importance attached to planulae in Lankester and Hyman's planula-acoel theories on the origins and relationships of the Metazoa², I studied the planula of the British solitary coral *Balanophyllia regia* (Anthozoa, Eupsammidae) from a flourishing "colony" established in an indoor tank. Sexual reproduction occurs in September and

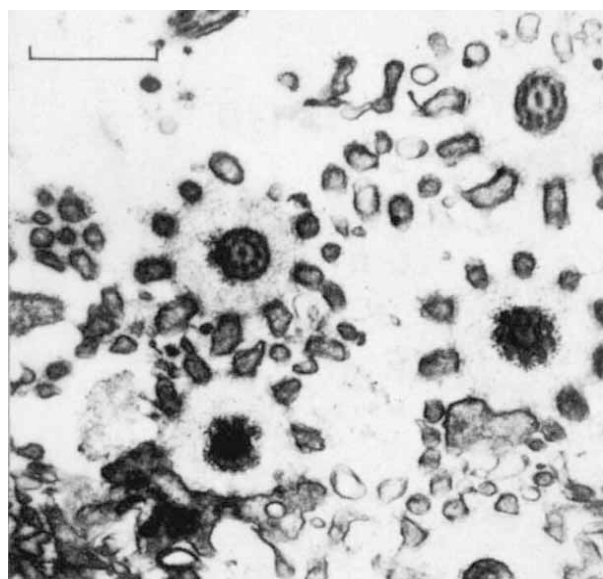


Fig. 1 Collar cells of planula ectoderm each with a single flagellum surrounded by 10–11 mucus-linked microvilli. Note vesicles at each side of the flagellum. (Index line 0.5 μ m.) Planulae were collected and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.7 M sucrose. After 1 h they were transferred directly to a cold 1% solution of osmium tetroxide in veronal acetate buffer, pH 7.2, containing 0.6 M sucrose and post fixed for 1 h. Material was embedded in 'Araldite' and double stained in uranyl acetate and Reynolds' lead citrate after sectioning.

free swimming orange yellow planulae are produced at this time.

In general morphology the planula resembles those hydrozoan planulae described by Van de Vyver³ and is covered by an ectoderm several cells deep (7.5 μ m). Surprisingly the majority of the surface ectoderm cells proved to be collar cells, comparable with those described for choanoflagellates⁴ and sponges^{5,6} but four types of gland cell, three kinds of nematocyte and spirocysts and nerve cells are also present. All the surface cells, with the possible exception of spirocysts⁷, bear a single flagellum. On the gland cell surfaces the flagellum is surrounded by randomly arrayed microvilli but in the collar cells it is surrounded by a ring of ten to eleven regularly arranged microvilli which form the collar (Fig. 1). Each microvillus is approximately 0.1 μ m in diameter and is linked into a ring by fibrous, mucus-like material, which extends inwards around the flagellum. The collars, which measure only 1.2 to 1.6 μ m in depth with an inside diameter of 0.6 μ m, are therefore less well developed than the collars of choanoflagellates and sponge choanocytes which are composed of a ring of 31 to 33 microvilli 6 to 7 μ m long⁴⁻⁶.



Fig. 2 LS of planula collar cell showing the accessory centriole and part of the rootlet and plaque with microfilaments. (Index line 0.5 μ m.)

The flagellum of the planula collar cell has the usual 9+2 tubule arrangement, is 20 to 25 μ m in length and has a diplosomal basal body with an accessory centriole. The basal body is produced into a 6 μ m long striated rootlet and attached to the accessory centriole is a plaque with a median dense thickening flanked by thinner lateral bands of dense material (Figs 2 and 3). The plaque connects with a fan of microfilaments joining to the cell boundaries on each side. An unusual feature of the collar cells is a dense inner cylinder with walls 40 nm thick that runs just inside the plasma membrane for 3 μ m stopping short of the termination of the striated flagellar rootlet (Figs 2 and 3). This cylinder may act as a support for the narrow neck regions of the collar cells. Flagella are occasionally found in the endoderm and may be those of differentiating gastrodermal cells. Many differentiating cell systems in mammals develop "flagella" in a non-dividing phase⁸.

Collar cells may not be present in all planulae and a published micrograph of a hydrozoan planula¹ suggests that this has a ciliated ectoderm. The stomodeal region of adult madreporian corals bears collar cells⁹ and in fact collar cells are much more common in the animal kingdom than is generally realized and may form a distinct cell type in forms as diverse as choano-

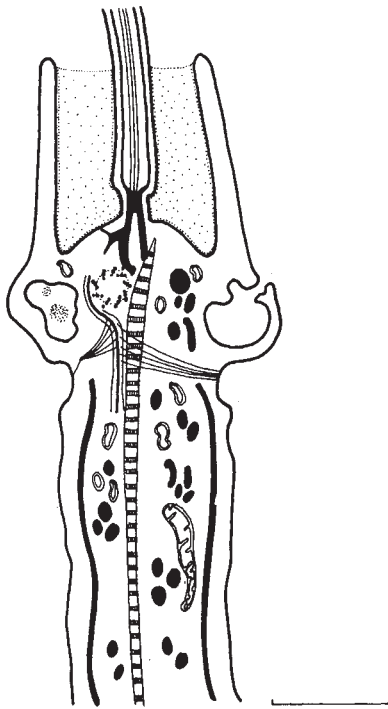


Fig. 3 Diagram of the outer part of a planula ectoderm cell showing mucus-filled collar and characteristic diplosomal basal body and rootlet structure. Vesicles are present at each side of this collar. (Index line 0.5 μm .)

flagellates and man. They may be present in the epidermis of a wide range of invertebrate larvae and have recently been described in the motile flagellate larva of *Rhabdopleura compacta*¹⁰ as well as composing the two "ciliary" rows on the tentacles of adult *Rhabdopleura*¹¹. Like other collar cell flagella those of *Rhabdopleura* have a diplosomal basal body. Echinoderm larvae and adults have collar cell epithelia^{12,13} and the tornaria larva of enteropneusts have been reported to have areas of epidermis with only one flagellum per cell¹⁴. These may be collar cells which are also present in the ventral collar region of the adult enteropneust *Harrimania*¹⁵. The gut of the brachiopod *Lingula unguis* has flagellate cells with an accessory centriole¹⁶ although details about the possible presence of a collar are not available. The endostyle of *Branchiostoma lanceolatum* also contains cells with a single flagellum and accessory centriole¹⁷ and true collar cells occur in the endostyle of *Botryllus schlosseri*¹⁸. The flagellum of hydrozoan cnidocils is surrounded by a collar of modified microvilli¹⁸⁻²⁰ and many sense cells are built on a collar cell plan ranging from hydrozoan independent mechanoreceptors²¹, to some sense cells in the tentacles of adult *Balanophyllia*, statocysts of hydrozoans²², sense cells in the epidermis of *Lumbricus terrestris*²³ and *Rhynchelmis*¹⁸ and sensilla of *Priapulus caudatus*¹⁸, chaetognaths¹⁸ and echinoderms¹⁸.

In vertebrates the rods and cones of amphibians and primates have a collar arrangement²⁴ with a flagellum incorporated into the rod and cone outer segments, an accessory centriole and a ring of twenty-seven microvilli (the so-called "dendrites") extending about half-way up the outer segment. Stretching a point, a case might just be made for considering that the hair cells of the vertebrate acoustico lateralis system were related to collar cells but with the "stereocilia" of the collar rearranged to one side of the "kinoflagellum"; the diplosomal condition persists in these cells²⁵.

The presence of ectodermal collar cells in a planula raises the possibility that they may be used for feeding during larval life although in view of the small depth of the collar and the large amount of larval yolk present this seems unlikely. Vesicles are present in the ectoderm cells (Figs 2 and 3) and open outside the collar, however, without using markers for

transport activity it is not possible to decide whether these are concerned with uptake or secretion. Their contents sometimes resemble dissociating dense granules which may be mucus secretion. The function of the nematocysts in the planula ectoderm is not known but if functional at this stage they are more likely to be used in defence or attachment than in feeding.

In an evolutionary context, the finding of flagellate collar cells in an anthozoan planula raises difficulties regarding the planula-acoel theory². It seems doubtful that if all planulae do have a collar cell ectoderm that a planula-like ancestor is likely to have given rise to an acoel-like form as the epidermis of acoels is ciliated and indeed "fundamentally" so as ciliated replacement cells form in the parenchyma and migrate out during epidermal regeneration²⁶. This is true also of triclads and monogeneans where a ciliated primary epidermis is formed early in their embryology^{27,28}. The finding of collar cells in acoels however would reopen the possibility of relationship. As collar cells occur in most major phyla of invertebrates as well as in vertebrates there is no *a priori* reason to suppose they may be absent from the platyhelminths. In fact, it has been suggested that flame cells are a modified type of collar cell¹². The indications that flagellate, often diplosomal, collar cells occur commonly throughout the animal kingdom may encourage speculation about the flagellate origins of the Metazoa²⁹ but deeper issues involve major decisions about the ability to distinguish properly between flagellum and cilium in the various contexts in which they seemingly appear.

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KATHLEEN M. LYONS

Department of Zoology,
King's College, London

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¹ Glätzer, K. H., *Cytobiologie*, **2**, 408 (1970).

² Hyman, L. H., *The Invertebrates*, **2** (McGraw-Hill, New York, 1951).

³ Van de Vyver, G., *Archs Biol., Liège*, **78**, 451 (1967).

⁴ Fjerdingstad, E. J., *Z. Zellforsch. mikrosk. Anat.*, **54**, 499 (1961a).

⁵ Rasmont, R., *Annls Sci. natn. (Zool.)*, **1**, 253 (1959).

⁶ Fjerdingstad, E. J., *Z. Zellforsch. mikrosk. Anat.*, **53**, 645 (1961b).

⁷ Westfall, J. A., *Amer. Zool.*, **5**, 377 (1965).

⁸ Rash, J. E., Shay, J. W., and Bieseke, J. J., *J. Ultr. Res.*, **29**, 470 (1969).

⁹ Goreau, T. F., and Philpott, D. E., *Expl. Cell Res.*, **10**, 552 (1956).

¹⁰ Dilly, P. N., *Marine Biol.*, **18**, 69 (1973).

¹¹ Dilly, P. N., *Z. Zellforsch. mikrosk. Anat.*, **129**, 20 (1972).

¹² Nørrevang, A., and Wingstrand, K. G., *Acta Zool.*, **51**, 241 (1970).

¹³ Cobb, J. L. S., *Jl R. microsc. Soc.*, **88**, 223 (1968).

¹⁴ Strathmann, R., *J. mar. Biol. Ecol.*, **6**, 109 (1971).

¹⁵ Nørrevang, A., *Nature*, **204**, 398 (1964).

¹⁶ Welsch, U., and Storch, V., *Einführung in Cytologie und Histologie der Tiere* (Fischer Verlag, Stuttgart, 1973).

¹⁷ Milanese, C. J., *Submicr. Cytol.*, **3**, 359 (1971).

¹⁸ Mattern, C. F. T., Park, H. D., and Daniel, W. A., *J. Cell Biol.*, **27**, 621 (1965).

¹⁹ Slautterback, D. B., *Z. Zellforsch. mikrosk. Anat.*, **79**, 296 (1967).

²⁰ Westfall, J., *Z. Zellforsch. mikrosk. Anat.*, **110**, 457 (1970).

²¹ Tardent, P., and Schmid, V., *Expl. Cell Res.*, **72**, 265 (1972).

²² Horridge, G. A., *Tissue and Cell*, **1**, 341 (1969).

²³ Knapp, M. F., and Mill, P. J., *Tissue and Cell*, **3**, 623 (1971).

²⁴ Brown, P. K., Gibbons, I. R., and Wald, G., *J. Cell Biol.*, **19**, 79 (1963).

²⁵ Roberts, B. L., and Ryan, K. P., *Proc. R Soc. Lond. B*, **179**, 157 (1971).

²⁶ Dorey, A. E., *Q. Jl Microsc. Sci.*, **106**, 147 (1965).

²⁷ Skaer, R. J., *J. Embryol. expl. Morph.*, **13**, 129 (1965).

²⁸ Lyons, K. M., *Parasitology*, **66**, 321 (1973).

²⁹ Remane, A. in *The Lower Metazoa*, edit. by Dougherty, E. C. (Univ. California Press, Berkeley and Los Angeles, 1963).

Thermoregulation in Dinosaurs

ANATOMICAL, physiological and ecological evidence has been assembled by Bakker¹ in an attempt to demonstrate that dinosaurs were endotherms. But critical parts of this evidence are less than convincing and may be interpreted differently.

GENERAL

Bakker suggests that dinosaurs were built for sustained locomotion at moderate speeds and infers from this that dinosaur energy metabolism was endotherm-like. But I consider it likely that dinosaurs, like terrestrial mammals, generally moved at low speeds and only reached maximum speeds in short bursts. The cheetah (*Acinonyx jubatus*) can attain 97 km h⁻¹ over a distance of 370 m (ref. 2), but soon slows down if the prey is not caught inside this distance. Thus the cheetah's top speed is maintained for about 15 or 20 s. Lizards are capable of fast running in anaerobic bursts lasting 20 s or so¹ and a similar mechanism could have permitted sophisticated cheetah-like predation in communities of ectothermic dinosaurs.

Bakker argues¹ that lack of body insulation, combined with endothermy, restricted dinosaurs to large size and observes that very small dinosaurs are unknown from deposits where mouse-sized salamanders, lizards and mammals are common. No dinosaur eggs are known to exceed a length of about 30 cm (ref. 3) and it is probable that the largest hatchlings were no bigger than a domestic cat (*Felis catus*). Dinosaur hatchlings equipped with the apparently lethal combination of small size, naked skin and an endothermic temperature regime could scarcely have survived to maturity. Yet dinosaurs did reproduce successfully, small individuals (even if juveniles) did exist, and evidence from "mummified" specimens indicates that dinosaur skins were naked^{3,4}. These facts seem to leave no other conclusion but that dinosaurs were ectotherms (unless one introduces the possibilities of parental care or of fossorial habits for hatchlings).

The mainstay of Bakker's argument¹ for endothermy in dinosaurs is the statement that endotherms "take prey at a rate an order of magnitude higher than ectotherms relative to body weight"—this premise being extended to analyse predator/prey ratios in dinosaur communities. Unfortunately, the ectotherm standard of comparison selected by Bakker is extremely unreliable. It consists of Auffenberg's estimate⁵ that the Komodo monitor (*Varanus komodoensis*) takes large prey about once a month, together with Bakker's own assumption that the weight of the prey is roughly half that of the lizard. From this it is deduced that the Komodo monitor consumes its own weight in prey every 60 d. But there is evidence^{5,6} that the Komodo monitor can take prey as heavy as itself and Bakker mentions¹ that the lizard may kill animals two or three times its own weight. This implies that the Komodo monitor ingests its own weight in prey every 10 or 15 d. To take the case of an endotherm it may be noted that the tiger (*Panthera tigris*) consumes a minimum of fifteen times its own body weight in prey each year⁷—that is, it ingests its own body weight in food every 24.3 d.

These figures seem to contradict Bakker's statement that endotherms take prey at a much faster rate than ectotherms. But these may be extreme cases and it seems safer to infer that there is no clear-cut difference between rates of predation in endotherms and ectotherms.

R. A. THULBORN

106 Park Hill,
Moseley,
Birmingham B13 8DB

Received November 2, 1972; revised May 31, 1973.

¹ Bakker, R. T., *Nature*, **238**, 81 (1972).

² Morris, D., *The Mammals* (Hodder and Stoughton, London, 1965).

³ Lapparent, A. F. de, and Lavocat, R., *Dinosauriens*, in *Traité de Paléontologie*, 5 (Masson, Paris, 1955).

⁴ Colbert, E. H., *Dinosaurs: Their Discovery and Their World* (Dutton, New York, 1961).

⁵ Auffenberg, W., *Anim. Kingd.*, **73**, 19 (1971).

⁶ Loveridge, A., *Reptiles of the Pacific World* (Macmillan, New York, 1946).

⁷ Schaller, G. B., *The Deer and the Tiger* (Chicago University Press, Chicago, 1967).

"Normal" Explanation of the Soal-Goldney Experiments in Extrasensory Perception

THE Soal-Goldney card-guessing experiments with the subject Shackleton consisted of forty sittings conducted between 1941 and 43 under the direction of S. G. Soal, then a lecturer in mathematics at the University of London¹. Elaborate precautions were taken against error and fraud. Many independent witnesses were called in. Copies of the records were made at each sitting and later independently checked. Above-chance scoring was achieved, in general, on the card one ahead of that being looked at, suggesting precognition. When the rate of guessing was speeded up, success shifted to the card two ahead. The statistical significance of the results was overwhelming. The series has long been quoted as a model of meticulous technique in experimental parapsychology and, for some people at least, has stood as a mainstay of the evidence for extrasensory perception.

In 1956, following a request by a sceptic to see the original records, Soal reported² that they had all been lost early in 1945—though the contemporary handmade copies were still available.

In 1960, Soal and Goldney reported³ for the first time that Mrs G. A., who took part as an "agent" in sittings 15 and 16, had told Goldney shortly after sitting 16 that she had seen Soal "altering the figures" during that sitting and, specifically, changing "1"s into "4"s and "5"s. Without informing Soal, Goldney and a colleague checked the original record but found no evidence of such alterations. Goldney then informed Soal who decided that the matter did not merit report. But Goldney kept a full contemporary record. Later, Scott heard of the affair, interviewed Mrs G. A. and threatened to publish an account. At this point the experimenters published their own account⁴.

In 1971, Medhurst⁴ attempted to identify the random number "target" sequence used in the Soal-Goldney experiments by a computer search based on Soal's detailed description of how he had derived the sequence. Had this succeeded it would have settled once for all any question of "altering the figures". In fact, the search failed completely, showing that either Soal's account of the method used in preparing the sequence was inaccurate or there had been widespread tampering.

Medhurst also showed that, if the "G. A. allegation" were true, it could only have been the target numbers that were altered and almost certainly at the time of the "check up", in which the guesses were compared with the targets.

We now describe the experimental arrangement. The "agent" (A) or sender sat behind a screen placed across a table. On A's side of the screen were placed, face downwards in a row inside an open box lying on its side, five animal picture cards: elephant, giraffe, lion, pelican, zebra. On the other side of the screen one of the experimenters (termed "E. A.") was in charge of the random number sequence. For each guess, E. A. held up the selected number to a window in the screen. The number N ($1 \leq N \leq 5$) instructed A to raise, and briefly look at, the N th card from the left in the row of five in the box. Meanwhile Shackleton sat in the adjoining room observed by an experimenter and recorded his guesses in the form of letter symbols: E, G, L, P or Z. The door between the rooms was left ajar so that he could hear E. A. call out the timing signal for each guess. After fifty guesses the order of the five cards in the box was recorded, the cards were reshuffled and a new run began. After a few runs there was a check up: the record of guesses was brought in and each guess was decoded into the corresponding digit 1 to 5 and entered opposite the appropriate target on the target record. The hits were then

counted. Sittings generally consisted of six or eight runs of fifty guesses each. Witnesses checked various parts of the procedure. Soal generally, but not always, took the principal role in the check-up.

The starting point of the present investigation was a cross-tabulation of target digit against the guess for sitting 16, taking account of the "+1 displacement" (guessing one card ahead). This is shown in Table 1. The guesses originally recorded as letter symbols have here been coded into digits according to the letter digit code used during each run.

Table 1 Target/Guess Matrix. Sitting 16, Successful Agents

Guesses:	1	2	3	4	5	Total
Targets						
1	20	12	22	5	0	59
2	13	20	12	20	13	78
3	11	20	19	12	15	77
4	5	9	11	33	6	64
5	5	10	10	7	26	58
Total	54	71	74	77	60	336

If target "1"s were changed into "4"s and "5"s in order to score hits, we would expect to find: (1) a deficit of total targets 1; (2) an excess of total targets 4 and 5; (3) a deficit specifically in the fourth and fifth cells in row 1; (4) an excess of hits on 4 and 5.

Effect (1) is found but the deficit is too small to explain the observed above chance score. Effect (2) is not found. Effect (3) is found at a high level of significance (probability of obtaining 5 or less in these two cells: 2×10^{-5}). Effect (4) is also found; because the null hypothesis is ESP, its significance depends on the ESP model assumed. We assume that the ESP scoring rates on the five different target letter-symbols may have varied randomly from run to run and independently of the digit-symbol. In the seven individual runs of Table 1 we then test the set of fourteen scoring rates on 4 and 5 against the fourteen rates on 2 and 3, using the *t*-test for difference of means (twenty-six degrees of freedom, single-tailed). We obtain $P = 2 \times 10^{-4}$. This probability is independent of that quoted above for the third effect.

Thus, in spite of overwhelming statistical support in tests (3) and (4), the overall target frequencies rule out the simple model of a change of target "1"s into "4"s and "5"s. An obvious modification of the model, however, would suppose that the target sequence was stacked in advance with an excess of "1"s and a deficit of "4"s and "5"s in order to conceal the manipulations planned. This would explain the results of all four tests above. More specifically, it predicts two further effects: (5) high scoring rate on 4, 5; lower but still appreciable rate on 1; no significant score on 2, 3; (6) in row 1, no tendency for cell 1 to exceed cells 2 and 3. In Table 2 we give the evidence on prediction (5).

Table 2 Sitting 16, Successful Agents

Symbol	Hits	Expected	Deviation
1	20	9.5	+10.5
2	20	16.5	+3.5
3	19	17.0	+2.0
4	33	14.7	+18.3
5	26	10.4	+15.6

For the final prediction (6), the figures for cells 1, 2 and 3 are, respectively, 20, 12, 22; these clearly fit the prediction.

Moreover, the model accounts for all the observations, including the "ESP" score, within the limits of reasonable sampling variation (taken as $\pm 2\sigma$). On the other hand, the data are not sufficiently plentiful for the success of these predictions to add significantly to those numbered (3) and (4).

We therefore searched for other occurrences of the highly specific effect (3) in the Soal-Goldney data. Two cases stood out very clearly: sitting 8 and the first three runs of sitting 17.

Table 3 gives the matrix for these runs pooled and Table 4 shows the analysis corresponding to Table 2.

Table 3 Target/Guess Matrix. Sitting 8 and First Three Sheets of Sitting 17

Guesses:	1	2	3	4	5	Total
Targets						
1	26	23	34	0	2	85
2	15	18	14	16	15	78
3	18	16	21	22	13	90
4	14	8	9	46	17	94
5	15	10	15	9	36	85
Total	88	75	93	93	83	432

"ESP" score: 147.

Most of the data for Table 3 were obtained with the counters method of target production. Because this gave slightly imperfect randomization we do not quote exact significance levels. But the pattern is clear: exactly the same effects (5) and (6) are seen as in sitting 16. These are fully independent of the search criterion (3) and amount to striking confirmation of the model.

Can these findings be attributed to capricious behaviour by ESP? Because ESP has no well established properties this alternative cannot be categorically refuted. But any such hypothesis has to face several difficulties. First, the observed anomalies appear in terms of digit symbols, whereas the ESP task was defined in terms of letter symbols, the code linking the two being changed every fifty guesses. Second, the anomalies appear with a consistent pattern in three different sittings and with two different agents. Third, the effects correspond to G. A.'s observation and are found in the sitting in which she reported it.

Table 4 Sitting 8 and First Three Sheets of Sitting 17

Symbol	Hits	Expected	Deviation
1	26	17.3	+8.7
2	18	13.5	+4.5
3	21	19.4	+1.6
4	46	20.2	+25.8
5	36	16.3	+19.7

The feasibility of making the supposed alterations unobserved depends on the disposition of the team of four persons during the checkup. In sitting 16, Soal's contemporary account reports that the one outside witness, B. P. Wiesner, decoded the guess letter symbols into digits and called these out to Soal, who then recorded them beside the appropriate target digit. Wiesner was checked by G. A. Soal was observed, according to the report, only by R. E., a regular "agent" in the experiments who later married Soal. Mrs Goldney was absent from this sitting, on war work. There is no evidence that Goldney was involved in any manipulation of the data: on the contrary, her evident desire to publish the full facts of the "G. A. allegation" argues against this.

In all three of the sittings mentioned the targets were recorded by Soal, either at or before the sitting. If he made his "1"s very short their alteration into "4"s and "5"s (even "2"s or "3"s) would have been possible.

Finally, the facts that the original records were lost and that Medhurst's computer search failed to identify the target sequence add some further support to the manipulation hypothesis.

The effects described are confined to three sittings. It could be argued that because the remaining thirty-seven sittings, which are independently significant, remain unexplained they must have involved ESP and hence that our explanation for the three sittings is highly improbable, for no one would fabricate data if genuine results were coming in. But conversely one could argue that the evidence for manipulation in the three sittings is overwhelming and hence the remaining results were very probably fabricated also, even if the method has not yet been identified. (Possible methods have been

suggested by Price⁵ and Hansel⁶, but see also Soal's replies^{7,8}. There may also be others never yet published.) In our view it has not been convincingly demonstrated that any "normal" explanation of the remaining sittings is excluded. The latter argument therefore seems the more rational one and we conclude that the present evidence substantially weakens the case for extrasensory perception, and in particular for precognition. A fuller account of these findings is being presented for publication elsewhere⁹.

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C. SCOTT
P. HASKELL

15 Sandwell Mansions,
West End Lane,
London NW6

Received March 1, 1973.

¹ Soal, S. G., and Goldney, K. M., *Proc. Soc. psych. Res.*, **47**, 21 (1943).

² Soal, S. G., *J. Soc. psych. Res.*, **38**, 216 (1956).

³ Soal, S. G., and Goldney, K. M., *J. Soc. psych. Res.*, **40**, 378 (1960).

⁴ Medhurst, G., *J. Soc. psych. Res.*, **46**, 39 (1971).

⁵ Price, G. R., *Science*, **122**, 363 (1955).

⁶ Hansel, C. E. M., *Proc. Soc. psych. Res.*, **53**, 1 (1960).

⁷ Soal, S. G., *J. Soc. psych. Res.*, **38**, 179 (1955).

⁸ Soal, S. G., *Proc. Soc. psych. Res.*, **53**, 43 (1960).

⁹ Scott, C., and Haskell, P., *Proc. Soc. psych. Res.* (in the press).

The Soal-Goldney Experiments

Scott and Haskell¹ have reported some formidable statistical evidence to support an allegation made by "G. A.". She acted as agent in two of forty card guessing experiments conducted between 1941 and 1943 (ref. 2). G. A. claimed that during sitting 16 she had seen Soal changing figures written in ink on a score-sheet: "1" into "4" or "5". There are patterns in the data for this sitting, and two other sittings, 8 and 17, which fit closely the hypothesis that the high scoring was due to this manipulation. Scott and Haskell argue that the patterns in question cannot plausibly be attributed to ESP. This is bound to raise doubts about all of Soal's contributions to psychical research; but there are some questions not discussed by Scott and Haskell which should be raised.

How reliable is G. A.'s testimony? There are inconsistencies in her statement and others have noted that she is not an accurate observer.

It is an understatement to say that there is no evidence that Goldney was involved in manipulation of the data. It was she who investigated G. A.'s allegation before disclosing it to Soal, and who disclosed from her contemporary records the specific claim whose implications Scott and Haskell have been testing. They describe her actions too briefly when they say: "Without informing Soal, Goldney and a colleague checked the original records . . .". Before sitting 17, Goldney wrote to Soal asking him to provide for her inspection all of the score-sheets for the previous sitting without giving any reason. She had not asked to see the score-sheets after the several earlier sittings from which she had been absent. It seems obvious that if Soal had employed the trick in question at sittings 8 and 16, Goldney's request would have been a danger-signal, and he would not have repeated this trick at sitting 17 in Goldney's presence. At sitting 17 the targets were determined by selection of counters, and to use the same trick Soal would have had to misrecord numbers during the experiment, entering a large number of "1"s written conspicuously differently from his normal "1"s. The latter are bold and full-length and could not be changed into "5"s without it being obvious. So I think we must suppose that if he practised this trick, Soal initially wrote short "1"s and during the decoding changed

all of them—to "4"s and "5"s or to his normal "1"s. Is it credible that Soal would have done this at sitting 17, having received from Goldney what if guilty he would have recognized as a danger-signal? He could not rely upon Goldney not to watch him during the decoding or to inspect the target list before decoding and notice an unusual shortness of all the "1"s. The fact that the statistical anomalies found in sitting 16 recur in sitting 17 seems to count rather strongly against the Scott and Haskell interpretation.

A computer analysis has shown that the high scores obtained in other sittings were not obtained by the trick in question, and has disclosed no other suspicious features in the data. In view of this, what one must weight against each other are: the improbability that Soal would have cheated in the three sittings on the assumption that he was getting genuine results in the other sittings; and the probability that the other thirty-seven sittings were faked by methods not yet identified, on the assumption that the evidence for manipulation in the three sittings is overwhelming. Scott and Haskell opt for the latter. But are they entitled to add: "Possible ways in which this [manipulation in the other sittings] might have been done have been suggested by Price and Hansel"? Surely not, for in order to explain several of the conditions in which high scores were obtained, Price and Hansel had to, and did, suppose that the "percipient", Shackleton, was an accomplice. But in that case it seems unintelligible that Soal should ever have resorted to the trick described by Scott and Haskell, as much safer methods of cheating were available. If Soal used the trick in question in the three sittings, we must assume that Shackleton was not an accomplice, in which case methods whereby Soal could have manipulated the results in all the other sittings have not been formulated. How, for example, can one explain sitting 28, when the target lists were prepared in Cambridge by C. U. Blascheck and posted to the observer, the late Professor C. A. Mace? He brought them to the sitting, handed one sheet at a time to Goldney, collected the guess-lists from Shackleton, and during the decoding and scoring stood behind Soal and Goldney and checked each step. Moreover, the experimental conditions in sittings 8, 16 and 17 were the same as in earlier sittings which had yielded high scores. Assuming, as Scott and Haskell do, that Soal had successfully practised other methods of manipulation in these experimental conditions, how is one to explain their assumption that Soal introduced such a risky method at sitting 8 which was attended by two independent observers who were present for the first time, and repeated it at sitting 17, when, apart from Goldney's presence, another independent observer was present for the first time? The observers at these experiments were free to, and encouraged to, observe whatever they wished.

I submit that there are difficulties in making psychological sense of the hypothesis adopted by Scott and Haskell. Such considerations are not, of course, conclusive, but they ought to be weighed before concluding that a scientist made a habit of cheating in his own experiments.

C. W. K. MUNDLE

University College of North Wales,
Bangor

Received March 27, 1973.

¹ Scott, C., and Haskell, P., *Nature*, **245**, 52 (1973).

² Soal, S. G., and Goldney, K. M., *Proc. Soc. Psychical Res.*, **47**, 21 (1942).

Reference Abbreviations

ALL abbreviations of references in *Nature* should now conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Authors submitting manuscripts are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Pursuit of Superalloys

The Superalloys. Edited by Chester T. Sims and William C. Hagel. Pp. xii+614. (John Wiley: New York and London, January 1973.) £13.25.

ONE of the significant gaps in metallurgical science is the inability to predict the structure and properties of alloys from the bonding characteristics of the constituent elements. In consequence, the technologist's search for materials that will meet engineering service needs has to be based on what one of the authors of this book has called enlightened empiricism. There is no better example in metallurgy of the successful application of this approach than the development of alloys for jet engine and gas turbine components. The scientific cynic might observe that the trade name for one of these alloys, 'Astroloy', is not too far removed from astrology, but it demands an activity more purposeful than star gazing to produce an alloy that will withstand a stress of 20,000 p.s.i. for 100 h at temperatures near 1,100° C. To be sure, dame fortune has smiled on the empiricist by giving him the remarkable γ' phase which has just the right characteristics, for example its accommodating tendency to increase its flow stress with temperature so that it peaks just in the range where the designer needs strength most. But the empiricist thrives on bits of luck of this kind.

The standard text by Betteridge on the Ni-based alloys has served the metallurgical community well since it appeared in 1959, but advances in the subject have made a new treatment timely. The present volume amply fills this role. Indeed it does more because it spreads its net more widely. It is admirably comprehensive, covering as it does the Ni, Fe, Co, and Cr-based alloys. Each of the twenty chapters is written by a different author. It is not easy these days for publishers to find a single author of appropriate stature to allocate the time needed to generate a definitive text on an important subject. The alternative, of breaking the story down into pieces under different authorship, can be a blueprint for disaster. But in the case of the volume under review, the editors are to be congratulated on their achievements in securing an amalgam of contributions of uniform style and treatment with a lack

of unnecessary duplication (aside from some reiteration of dislocation strengthening theories) and with a generally attractive presentation.

The metallography of these alloys has a strong appeal to anyone with artistic sensibility, and a rich store of inspiration for fabric and wallpaper designers resides in an atlas of microstructures of the superalloys. The illustrations in the text do more than justice to these structures. The student will find them admirably chosen to bring out clearly the characteristic morphology and distribution of many phases that can arise. He will also wish to equal in his own metallography notebook the quality of the carefully sketched diagrams of important structures.

The volume begins with an excellent introduction that lays out the stringent service requirements for materials for engine components, and reviews briefly the range of alloys available and the properties currently achieved. There follow detailed accounts of the metallurgy of the Ni-based alloys, Ni-Fe alloys, Co-based alloys and Cr-based alloys with a separate chapter on dispersion strengthening. Chemical stability, protective coatings and all aspects of processing are thoroughly dealt with. This readable and comprehensive review of the current state of the art of development of alloys that must surely be regarded as the metallurgical superstars prompts speculation on the scale of advance over the next decade, when the time might be ripe for a new text. It is difficult to believe that a dramatic upward extension of the useful temperature range of the Ni alloys can be brought about, and the fluctuating fortunes of the Cr alloys give them a none-too-bright future through their hereditary fault of being born body-centred cubic, with the associated tendency to brittleness and a distressing propensity to form assignments with nitrogen.

But the enlightened empiricists will not be deterred by such depressing talk of diminishing returns. Metallurgy has wrought remarkable triumphs in its time and restlessly and optimistically strives to attain new heights. Those who are minded to join the fray and try their hand at improving the superalloys would be well advised to consult the present volume first, for in it they will find a clear, concise and authoritative treatment of the present state of the art,

which is the springboard from which further advances will be achieved. Even at £13.25 the book could be a rewarding investment. It should also be read by anyone, technologist, engineer, student or academic, whose education would be the poorer if he (or she) did not have some acquaintance with this exciting part of modern alloy development.

K. M. ENTWISTLE

Psychocomputation

The Computer in Psychology. Edited by Michael J. Apter and George Westby. Pp. xvi+309. (John Wiley: London and New York, March 1973.) £5.50.

THIS book is a compendium of ten essays by six different authors, loosely bound together by a foreword and a preface, to the effect that students of psychology ought to know something about computers and their present relevance to psychology. It is not, perhaps, a serious criticism of the book to say that the subject matter of each chapter might better be studied in a monograph devoted to that subject; there is much to be said for bringing together in one place a set of introductory articles to help the hard-pressed student identify what he ought to know about computers for his purposes. One can imagine an enterprising publisher producing a whole series of books under the general title "The Computer in (Scient)ology" where the science in question could be anything from chemistry to literary criticism. The first two chapters could be common to all the books, and would be (as they are in this book) entitled "An Introduction to Computers" and "An Introduction to Programming" respectively. The third and fourth chapters on the use of computers for doing experiments would also have their counterparts in any such book, as would much of the material of part 2, entitled "Areas of Application".

There is, however, a special reason why psychologists should be interested in computers—a reason which does not apply to the other sciences where computers are found so helpful. The reason is that the human brain, which mediates the behaviour and generates the thoughts which psychologists study, is itself a computer—indeed by far the

most sophisticated computer known to exist. Its principles of operation are obviously very different in many respects from those of man-made computing systems; but it is already clear that comparisons between the two can provide an immense intellectual stimulus to the precise formulation of psychological theories. The most interesting parts of *The Computer in Psychology* are those which recognize this fact explicitly: chapter 5, by Michael Apter, on "The Computer Modelling of Behaviour", and chapter 7, entitled "The Computer in the Psychology of Language", both emphasize the value of programmable theories of language and other types of behaviour; the same spirit informs the chapter by James Robinson on "The Computer in Clinical Psychology". The last chapter of the book, entitled "The Computer in Education and Training", is a business-like account of the present state of computer-aided instruction—an art which is still in its infancy, but is likely to make rapid progress once we understand our own thought processes a good deal better.

CHRISTOPHER LONGUET-HIGGINS

Birth of the Stars

Stellar Evolution. Edited by Hong-Yee Chui and Amador Muriel. Pp. xiv+812. (MIT: Cambridge, Massachusetts and London, June 1972.) \$15.

THE evolution of stars is the branch of astrophysics which we think we understand best. For a long time we have had a good qualitative understanding of how observed properties of stars can be explained in terms of their mass, chemical composition and age, provided only that nothing causes them to depart too far from spherical shape. Our understanding is best in the early stages of evolution, when a star radiates energy released by the conversion of hydrogen into helium in its interior, although the process of star formation is still imperfectly understood.

There are both mathematical and physical difficulties in attempting to understand the final stages of stellar evolution, but they have been studied much in recent years for several reasons. One is concerned with the origin of the chemical elements. If nuclear reactions in stars make a significant difference to the overall chemical composition of the Galaxy, it is necessary to discover what nuclear reactions occur in individual stars and how much matter they expel into interstellar space. A second reason for studying late stages of stellar evolution is that some of the most exotic objects in the Galaxy are believed to be formed then; these include neutron stars, which are believed to exist in pulsars, and the so far unconfirmed black holes.

Although we feel that we understand the early stages of stellar evolution, this belief has been shaken recently by the failure to detect a significant number of energetic neutrinos released by nuclear reactions near the centre of the Sun. The number detected in a careful experiment is fewer than predicted by any apparently acceptable model of the Sun. Even though theory and observation may be reconciled eventually, we are forced to realize that it may be far easier to understand two observed surface properties of a star (luminosity and surface temperature) than to predict its complete interior structure.

This book contains the edited texts of lectures given at the 1969 Summer Institute for Astronomy and Astrophysics at Stony Brook, New York, which was devoted to the study of stellar evolution. I am usually very critical of books which contain lectures by a wide variety of authors, because they lack the coherence of a book by a single author. However, this book is certainly very good of its type, dealing not only with the well understood aspects of stellar evolution theory but also with problems in an active stage of development.

There are several articles on early stellar evolution. The book starts with a lengthy and clear account of normal stellar evolution by Iben. A valuable feature of this article is his regular use of order of magnitude physical arguments and there is also a very detailed discussion of the solar neutrino problem as it appeared in 1969. Demarque and Stothers discuss the early evolution of stars of ordinary mass and very high mass, respectively, and the main physical processes in stellar interiors are described by Carson (opacity), Spiegel (transport mechanisms) and Truran (nuclear reactions).

As I mentioned earlier, there has recently been much study of the late stages of stellar evolution, particularly those leading to supernova explosions and their after effects. Paczynski discusses one of the possible supernova models and Colgate and McKee explain why, in their view, the explosion of a star leads to a very high luminosity and not merely a high kinetic energy. White dwarfs are certainly an end point of stellar evolution. They used to be thought to be remnants of supernova explosions but that seems less likely now; they are discussed in great detail by Ostriker. Neutron stars and pulsars, which are almost certainly supernova remnants, are the subjects of chapters by Cameron and Chiu, and Truran describes the nucleosynthesis which may accompany a supernova explosion.

Another valuable series of lectures are those by Mestel on problems of

stellar rotation, magnetic fields and star formation. There is also a group of chapters on stellar stability and mass loss from stars; stellar stability by Baker, variable stars by Christy, close binaries and mass exchange by Paczynski, novae and shell instabilities by Rose. In addition there are a few papers in categories of their own; relativistic stars by Thorne, intense magnetic fields by Chiu and Canuto, stellar coalescence by Cameron and horizontal branch stars by Philip.

Most of the subject of stellar evolution is covered by the chapters I have mentioned. It is impossible to discuss them all critically in a brief review but I am impressed by their general quality. Although the subject has developed since 1969, this is still a worthwhile publication at a reasonable price. I have only one minor grumble; the publishers say that they photocopied typescript to enable the book to appear as soon as possible but even so it came out three years after the lectures.

R. J. TAYLER

Insect Development

Developmental Systems: Insects. Edited by S. J. Counce and C. H. Waddington. Volume 1: Pp. xiii+304. Volume 2: Pp. xiii+615. (Academic Press: New York and London, December 1972.) £6.50 and £10.

INSECTS have provided and will continue to provide ideal material for the study of all the processes of growth and development. Their size is such that the relations between the final form and the cells by which it is built are often wonderfully clear to the observer. This does not make the process of growth intelligible—but it constantly inspires the investigator with the feeling that given a little more knowledge everything could be understood. The purpose of the book under review was to put a detailed account of what has been achieved with this material before developmental biologists in general. It would have been possible to provide an illuminating volume that would have covered the entire theme in imaginative outline with details of selected topics where these might be particularly instructive. But the ten contributors to *Developmental Systems: Insects* seem to have been invited to prepare fully documented and detailed reviews of their topics. The result is that the book has burst its bounds and divided amitotically into two volumes, and at the same time the topics covered have had to be severely restricted. In the event, however, we have a series of highly competent reviews of certain fields of insect development.

Oogenesis in insects, reviewed by A. P. Mahowald, has long been an area

of polemical discord. Solid advances have been made in recent years, notably with the electron microscope; and it is interesting to find that the processes of transfer and synthesis of yolk components are so varied that virtually all the disputed claims of the past can now be justified. Three sections, development of apterygote insects by C. Jura, and of hemimetabolous and holometabolous insects by D. T. Anderson, contain representative descriptive accounts of all the classic embryology of insects. It is valuable to have all this material so brought together. The final chapter of Volume 1 deals with polyembryony in insects by O. M. Ivanova-Kasas—a fascinating subject now fully reviewed for the first time.

The book as a whole is dedicated to Professor Friedrich Seidel who, with his numerous students, has been the chief architect of experimental embryology in insects. Volume 2 opens with a 150-page chapter in which S. J. Counce reviews in detail "the causal analysis of insect embryogenesis". It must be admitted that little has been added to the basic principles since Seidel's classic work in the 1920s and 1930s. It is still a matter of "centres" the physiological activities of which are very uncertain. The succeeding section by P. A. Lawrence is less concerned with completeness than with ideas. It provides a stimulating discussion of gradients in relation to differentiation. C. M. Child battled for years for recognition of the importance of gradients—but without much success. Gradients of what? As the review by Lawrence makes clear, it is possible to define the laws of gradients without knowing the true nature of the phenomenon. To discuss gradients of an imaginary something is no more and no less an invocation of the occult than is the idea of gravity—which, after nearly four hundred years, is still a useful concept.

Following upon the striking successes of E. Hadorn's researches, the study of the imaginal disks of *Drosophila* is a popular theme. It is well reviewed here by W. J. Gehring and R. Nöthinger. W. W. Doane contributes 175 pages of text with nearly 1,400 references on the role of hormones in insect development. My first reaction was that everything had been swept in, grain and chaff together. But having read the whole section I have to admit that, although exhaustive and somewhat exhausting, it is informed throughout by critical judgment. The book ends with a chapter by the second editor C. H. Waddington on the morphogenesis of patterns in *Drosophila*. This makes interesting reading, for much is based on Waddington's own early work and leads on to more recent developments from it.

Altogether this is a book that con-

tains a great deal that is of value—but it does not have the unity of a work of art.

V. B. WIGGLESWORTH

Fruit Fly Populations

Behavioural and Ecological Genetics: a Study in Drosophila. By P. A. Parsons. Pp. vii+223. (Clarendon: Oxford; Oxford University: London, March 1973.) £6.50.

THIS volume comprises a survey of recent work, with various species of *Drosophila*, which has provided evidence of genetic differences in behaviour and reaction to environmental conditions. Both field and laboratory studies are covered. The behaviour section deals with such topics as the effects of polygenic variation on behaviour traits as indicated by diallele crosses or selection for changes in geotaxis or mating speed. The second part is concerned with the evidence for genetic variation within and between populations in reaction to different kinds of environmental stress, laboratory studies on competition and the effects of genetic heterogeneity of various kinds on population growth, inversion polymorphism, the characteristics of marginal and central populations and also the contribution of behavioural and ecological differences to genetic isolation. The last section, although referred to as a synthesis, continues the story in terms of species distribution, interspecific competition and a discussion of the "adaptedness" of populations.

The literature survey is quite complete up to mid-1971, so this book serves a useful purpose as a source of references and a guide to the wide variety of genetic problems in ecology and behaviour which can be profitably studied with the aid of appropriate species of *Drosophila*.

The treatment of the material suggests that summaries of papers have played a large part in the compilation. The author very properly stresses the difficulties in defining adequate measures of the fitness of individuals and the "adaptedness" of populations, but one could go further. Given the frequent underestimation of the ecological complexity of the laboratory *Drosophila* culture, the often limited generality of measures of competition, inter-genotype interaction and so-called facilitation, which are all readily influenced by the particular conditions of the laboratory test as well as the genotypes which happen to be available, together with the frequent absence of adequate tests of repeatability, it would seem reasonable to encourage a robust scepticism, especially with regard to extrapolation to the even less understood natural situations. One suspects that, whatever the fashionable model, there would be no shortage of data

which appeared to support it. When the interpretation of many experiments in ecological genetics is debatable it is premature to expect any author to provide a confident synthesis. But in that case it is all the more necessary to strike a probing, analytical attitude, otherwise the inexperienced reader will certainly be tempted to overestimate the number of problems which have been satisfactorily disposed of. It is probably significant that the book begins and ends with a reference to the remarkable *Drosophila* fauna of Hawaii which is beginning to yield up some of its evolutionary secrets as a consequence of a comprehensive, comparative study of many aspects of ecology and behaviour in both laboratory and the field. Integrated studies of this kind are needed for other groups of species which occur nearer home.

F. W. ROBERTSON

Neutron Scattering

Chemical Applications of Thermal Neutron Scattering. Edited by B. T. M. Willis. Pp. xiv+312. (Oxford University: London, March 1973.) £9.50.

ALTHOUGH the opportunities for carrying out experiments in neutron scattering are few, and likely to remain so because of the expense of intense sources of neutrons, it is evident from this book that the rewards for perseverance are immense.

Dr Willis's book brings together twelve chapters written at an advanced level by individual experts. The first two consider basic theory and experimental technique and the succeeding ten provide a very up-to-date picture of what can be achieved by neutron beam studies in different branches of "chemistry". Among the topics are molecular spectroscopy, polymers, liquid, glasses and non-stoichiometric compounds. It might be argued that some of these chemical applications have a decidedly physical flavour, but it is clearly evident that neutron scattering, like other good physical techniques, has now reached the stage at which it is in essential use by chemists. In view of the fundamental possibilities which the neutron provides this use is already overdue and it is often forgotten that, as a diffraction technique, neutrons are now half as old as X rays.

The book arises from a series of lectures given at a Harwell Summer School and the editor is to be congratulated on having welded together his contributions with a coherence which is unusual in this kind of compilation. The symbolism and mode of theoretical approach which is set by the basic introductory chapter is faithfully followed by the later contributions. This greatly increases the value of the book to the non-expert and will permit him to learn a good deal

from a direct straight reading. Two short appendices which explain the much-used concepts of the Dirac δ -function and the Fourier transform are very worthwhile. Each chapter is well referenced up to 1971, and occasionally into 1972, and there are author and subject indices.

The book appears at an opportune moment when the Science Research Council has taken a third share in the Franco-German high-flux reactor at Grenoble and will have served a valuable purpose if it encourages British chemists to make use of the instruments which are now available to them there.

G. E. BACON

Electrons and Holes

Point Defects in Solids. Volume I. General and Ionic Crystals. Edited by James H. Crawford, jun., and Lawrence M. Slifkin. Pp. xv+556. (Plenum Press: New York and London, 1972.) \$43.

THERE are several stages in the excitation of electrons in ionic crystals, ranging from the closely bound electron-hole pair (exciton) to the complete separation of electrons and holes. In the pure crystal the electron, if it is free for sufficient time, may contribute to conduction. The transport occurs, however, not by a relatively free and simple particle, but by a "quasi-particle" comprising the electron and its associated cloud of polarization. The properties of polarons—electrons plus a pattern of ionic displacements—require a detailed knowledge of the interaction between electronic and vibrational excitations.

At sufficiently low temperatures the hole may immobilize itself by the polarization which it creates, producing a characteristic ESR spectrum. The whole phenomenon of the self-trapped hole is the more readily observed if there are deliberately added electron traps to give a sufficiently hole lifetime.

Localization of electrons and holes also occurs at a wide variety of crystal imperfections—point defects, impurities and complexes involving either or both categories. The spectroscopy of these centres has reached a high level of sophistication. It covers electronic, spin and vibrational excitation of the centre and their interaction with the rest of the crystal. The techniques include those of unidirectional externally applied perturbations, such as electric, magnetic and stress fields, to determine the symmetry of the wave functions—a crucially important aspect of relating the structure of the defect to its atomic environment. In addition oscillatory perturbations have allowed complex modulation spectroscopy to be used both for microwave and optical spectra.

The electron centres are probably best known. They include the F centre

(electrons localized at halogen vacancy) and the M centre (electrons localized at an associated pair of halogen vacancies). The hole centres were first thought to be complementary but have turned out to be quite different. They occur with extra halogens and uniaxial relaxations along the rows of halogen ions; the entity is a many-body collation of relaxed ions.

It is clear from the foregoing that the control of defects must be important. It is achieved by heat treatment to produce departures from stoichiometry, by the addition of differently charged substituted (aliovalent) ions (for example Sr^{2+} for Na^+ produces a further Na^+ vacancy) and by controlled irradiation. The defects and complexes have symmetries because of where they are situated in the lattice and these may be studied by relaxation methods—in an electric field (dielectric relaxation) or in a stress field (anelastic relaxation). These relaxation processes of complexes provide the microscopic mechanisms for larger scale mass transport (diffusion) studies.

Electrons trapped at such defects may give rise to very sharp absorption and emission spectral lines, as well as the associated (commoner) bands. The zero-point uncertainties in the electronic states give a probability of a transition unbroadened by lattice vibrations (the zero-phonon spike, physically similar to Mössbauer γ -ray spike and band).

Underlying the whole subject is the knowledge of defect motion from the study of ionic conductivity at high temperatures, which is really where it all began. The ions needed space in which to move and intrinsic high temperature defects were adduced by Frenkel and Schottky to provide that space. The mass diffusion already referred to is also closely related to the ionic conduction process.

The subject is, therefore, a fascinating interplay of widely differing experimental and theoretical techniques. The present volume—the first of three setting out to provide a comprehensive discussion of the state of knowledge about point defects in solids—deals primarily with the subject outlined above, with a chapter on the statistical thermodynamics of defect formation and motion which provides a background for the whole series.

From the early days of the Göttingen work, advances have occurred, time and time again, as a result of carefully conceived experiments carried out with great sophistication on well characterized and controlled crystals. This comes through very clearly in the different sections. The sympathetic support of a number of theoretical physicists has led to the standard modern theory having been part of the jargon of the subject for a long time. An in-

creasing physics interest in the field, apart from the desire comprehensively to understand a few simple crystals, is that it provides well defined experimental situations where theorists can test many-body approximations in systems with chosen degrees of coupling.

There is a remarkable continuing interest in this classic field of modern solid state physics and the present—expensive—volume will be a necessary addition to any library concerned with physics. The articles are well and authoritatively written and I would express only minor disappointment at the omission from so fine a volume of a discussion of the optical pumping *via* excited F-centre states and of the tunnelling phenomena between F centres.

E. W. J. MITCHELL

Medicinal Plants

Pharmacographia Indica. By William Dymock, C. J. H. Warden and David Hooper. Pp. 546. (The Institute of Health and Tibbi Research under the auspices of Hamdard National Foundation: Pakistan, 1972.) Pak. Rs. 220; US\$20.

THIS book has now been reprinted largely under the aegis of UNESCO following an international symposium on medicinal plants held in Peshawar in 1960. This is still the classical work of reference on the medicinal plants of the Indo-Pakistan subcontinent. Originally in three volumes, the *Pharmacographia* has now been produced in one volume by offset printing and the production is very good. Dymock and his colleagues, Warden and Hooper, produced these volumes in 1890 largely based on the smaller work relating to the medicinal plants of Western India and the drugs sold in that great drug market of the East, Bombay, produced in 1883 by Dymock. Dymock and his co-workers produced a great work of scholarship on the drugs and medicinal plants of India which up until then had been scattered through books and periodicals in many languages, but in addition where they appeared deficient in information they performed original investigations particularly towards elucidating the chemical composition and the physiological action of the plants and drugs. In this highly scientific work, however, Dymock felt that certain plants long used in India and of historical and mythological interest also had to be listed although they had little or no medicinal activity. The book is therefore a fascinating hotch-potch of superstition and science and not a little poetry. It is, of course, the intention to bring the work up to date but the editors and publishers are to be congratulated on bringing a rare work into more general circulation.

EDWARD HITCHCOCK

CORRESPONDENCE

Lunar Conductivity

SIR,—Your geomagnetism correspondent¹ commented on the problem of lunar conductivity where he reviewed the current status of the problem of inversion of lunar magnetometer data into a profile of the bulk electrical conductivity. His statements tend to centre only on the recent work of Hobbs² where the uniqueness of lunar data is discussed in terms of the Backus–Gilbert resolution criterion^{3–5}. Both the comments in *Nature* and those of Hobbs are based on our very first preliminary report⁶, now over two years old, of the lunar transfer (response) function and the inversion resulting from these data. Thus, the comments in *Nature* completely bypass our later reports^{7,8} which extend the data both in accuracy and bandwidth as well as making use of a theory in the inversion which includes higher magnetic multipoles of the fields. The discussion in *Nature* and that by Hobbs, both of historical interest only, unfortunately do not give the background upon which our publication of the conductivity spike was based. In that work, there was some evidence that the transfer function “rolled over” beyond a frequency of about 0.01 Hz. In addition, the basalt lunar rock conductivity work published by Nagata *et al.*⁹, when used together with published values of typical ultrabasic rock conductivity, provided a smooth temperature profile. The latter must be regarded as a key element in our early conclusion since the existence of the “rollover” phenomenon forced our solutions towards a “spike” irrespective of a variety of starting conditions in the iteration. The importance of this can best be understood when it is noted that in Hobbs’s calculations the iterations and resolution tests are restricted to an upper bandwidth limit of 0.01 Hz, exactly where difficulty ensues in fitting a monotonic conductivity function to the data. The spike can thus be avoided by the use of dipole (or long wavelength) theory, and the combination of ultrabasic and Nagata-basalt conductivity functions which led to the conclusion that a conductivity “spike” exists in the lunar mantle.

If Hobbs had used our later data, published well before his paper and over the full frequency range, he should have reproduced our results showing that higher order magnetic multipoles are responsible for the prominent “roll-

over” in the lunar transfer function, that the higher order theory is indeed required in iteration, and that we had already produced models which are bounded by the Backus–Gilbert criterion.

The latter is no panacea for testing the uniqueness of lunar magnetometer data. Indeed, for the very limited resolution presently available, a strong argument can be made that better intuitive physical insight is gained by considering a spectrum of electrically equivalent models, just as we did. Further, Backus–Gilbert calculations carried out by us even earlier than the generation of our present model spectrum (unpublished) show no disparity with the set consisting of two-layer, three-layer, four-layer, core-plus-current layer, and two-current layer models as determined by the residual mean square error difference between model forward calculations and the data, and the mean square average noise in the data itself^{7,8}.

The Backus–Gilbert criterion, exquisite though it is, provides no intrinsic measure of confidence in the models beyond what we already have calculated, considering the overall quality of the data, and perhaps even more critical, the “goodness” of the theory which will always remain an external constraint upon the final model attained. A more careful reading of the literature would show that, though the time for use of the Backus–Gilbert criterion may be fast approaching, there is still considerable work to be done to improve the inversion by inclusion of the full higher-order theory arising from cavity asymmetry, short wavelengths in the solar wind, separation of data according to propagation vector direction, and a host of smaller but nevertheless still germane problems. In short, Backus–Gilbert tests are not required to show that the “spike”, though still with us, is non-unique. Finally, the Kuckes two-layer model¹⁰, or indeed any two-layer model, has already been shown by us to lie outside the error tolerance of the data⁸.

Yours faithfully,

C. P. SONETT

Lunar and Planetary Laboratory,
University of Arizona,
Tucson, Arizona 85721

¹ Anonymous, *Nature*, **243**, 265 (1973).

² Hobbs, B. A., *Earth Planet. Sci. Letters*, **17**, 380 (1973).

³ Backus, G. E., and Gilbert, J. F., *Geophys. J.*, **13**, 247 (1967).

⁴ Backus, G. E., and Gilbert, J. F., *Geophys. J.*, **16**, 169 (1968).

⁵ Backus, G. E., and Gilbert, J. F., *Phil. Trans. Roy. Soc., A*, **266**, 123 (1970).

⁶ Sonett, C. P., Colburn, D. S., Dyal, P., Parkin, C. W., Smith, B. F., Schubert, G., and Schwartz, K., *Nature*, **230**, 359 (1971).

⁷ Sonett, C. P., Schubert, G., Smith, B. F., Schwartz, K., and Colburn, D. S., in *Proc. Second Lunar Sci. Conf.* (edit. by Levinson, A. A.), **3**, 2415 (MIT Press, Cambridge, Massachusetts, 1971).

⁸ Sonett, C. P., Smith, B. F., Colburn, D. S., Schubert, G., and Schwartz, K., in *Proc. Third Lunar Sci. Conf.* (edit. by Heymann, D.), **3**, 2309 (MIT Press, 1972).

⁹ Nagata, T., Rikitake, T., and Kono, M., *COSPAR*, **10** (1970).

¹⁰ Kuckes, A. F., *Nature*, **232**, 249 (1971).

Missing Magnitudes

SIR,—The recent article on polypeptide configurational kinetics entitled “The Missing Magnitudes” (*Nature*, **243**, 186; 1973) is, we feel, premature in its conclusions. An explanation, based on sample polydispersity, of differences between the two ranges of molecular times inferred from experiments is inadequate. One of us has recently fractionated poly- γ -benzyl-L-glutamate (J. B. Milstein and J. A. Ferretti, *Biopolymers*, in the press). The n.m.r. spectra of sharp fractions exhibit α -C-H proton peak doubling, in direct contradiction of results by Nagayama and Wada (*Chem. Phys. Lett.*, **16**, 50; 1972; and *Biopolymers*, in the press). Thus the problem remains unresolved.

We assert that an explanation based on polydispersity cannot logically be extended to comprehend the experimental chain length dependence of the double peaks. Therefore, we would still maintain that the origin of the n.m.r. double peaks lies in the existence of a slow nucleation time; in terms of our models this is the time for forming an initial α -helix from an all random coil molecule.

Even if this is not the finally accepted explanation of the n.m.r. behaviour, a treatment of helix nucleation in chain molecules is requisite to a detailed understanding of conformational kinetics. Theories developed by Miller (*Macromolecules*, **6**, 100; 1973) and by Jernigan, Weiss and Ferretti (*Macromolecules*, in the press) should have applications to time dependent properties

other than n.m.r. behaviour. Self-organization in proteins may possibly be understood through more developed versions of such theories.

Yours faithfully,

JAMES A. FERRETTI
ROBERT L. JERNIGAN

Physical Sciences Laboratory,
Division of Computer Research
and Technology,
National Institutes of Health,
Bethesda, Maryland 20014

W. G. MILLER
Department of Chemistry,
University of Minnesota,
Minneapolis, Minnesota 55455

Announcements

Miscellaneous

Professor J. R. Vane, Royal College of Surgeons, has been appointed a director of the Wellcome Foundation.

Errata

In the article "Distance Measurements across the Heimae Eruptive Fissure" by J. Brander and G. Wadge (*Nature*, **244**, 496; 1973), line 6, paragraph 4, should read "... lines, we found that $E_1 = 336$ p.p.m. and $E_2 = -420$ p.p.m., ..."

In the article "Accurate Position of GX5-1 from Lunar Occultations", by J. A. Hoffman, P. J. N. Davison and L. V. Morrison (*Nature*, **244**, 347; 1973) the list of authors should have read in alphabetical order.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

SI: The International System of Units. Edited by Chester H. Page and Paul Vigoureux. (Translation approved by the International Bureau of Weights and Measurements of its publication "Le Systeme International d'Unites".) (Department of Trade and Industry: National Physical Laboratory.) Pp. ix+47. (London: HMSO, 1973.) 63p net. [256]

Energy Policy, Vol. 1, No. 1, June 1973. Edited by Dr. John A. G. Thomas. Pp. 1-88. Published quarterly. Annual subscription (four issues) £14; \$36.40. (Guildford, Surrey: IPC Science and Technology Press, Ltd., 32 High Street, 1973.) [256]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 265, No. 871: The Ordovician Fossil *Lagynocystis pyramidalis* (Barrande) and the Ancestry of Amphioxys. By R. P. S. Jeffries. Pp. 409-469+plates 37-46. (London: The Royal Society, 1973.) £3.30; \$9.25. [256]

Department of Education and Science. Provincial Museums and Galleries. (A Report of a Committee appointed by the Paymaster General.) Pp. vii+92. (London: HMSO, 1973.) £1 net. [256]

Ministry of Agriculture, Fisheries and Food. Fisheries Laboratory, Lowestoft. Report of the Director of Marine Fishery Research for the years 1969-1971. Pp. 81. (Lowestoft, Suffolk: Ministry of Agriculture, Fisheries and Food, Fisheries Laboratory, 1973.) [256]

Department of the Environment. Fourteenth Progress Report of the Standing Technical Committee on Synthetic Detergents. Pp. v+29. (London: HMSO, 1973.) 30p net. [266]

A Maturing Democracy: The Role of Broadcasting. By Charles Curran. (Speech given to the National Liberal Club, London, 14 March 1973.) Pp. 23. (London: BBC, 1973.) [266]

Department of Trade and Industry. National Physical Laboratory—Materials Group Research 1972. Pp. xi+146. (London: HMSO, 1973.) £1.20 net. [266]

The Cotton Silk and Man-made Fibres Research Association, Shirley Institute. Report and Accounts, 1971/1972. Pp. 35. (Manchester: Shirley Institute, 1973.) [276]

Cancer Research Campaign. 50th Annual Report, 1972. Pp. 148. (London: Cancer Research Campaign, 2 Carlton House Terrace, 1973.) [276]

Centenary of the Huxley Building: Personalities and Events in the History of Imperial College. By Dr. C. J. Whitrow. Pp. 23. (London: Publications Officer, Imperial College, 1973.) [276]

Proceedings of the Royal Irish Academy. Vol. 73, Section B. No. 7: The Effects of Contraction and Ischaemia on Creatine Phosphate and Adenosine Triphosphate in *M. semitendinosus* of the Pig. By J. V. McLoughlin, P. J. V. Tarrant and M. G. Harrington. Pp. 95-108, 21p. No. 8: The Sub-Old Red Sandstone Surface in Southern Ireland. By R. J. P. Doran, C. H. Holland and A. A. Jackson. Pp. 109-128+plates 9 and 10. 39p. (Dublin: Royal Irish Academy, 1973.) [296]

Courtaulds. Report and Accounts 1972/1973. Pp. 24. (London: Courtaulds Ltd., 1973.) [296]

Republic of Ireland. Annual Report of the National Drugs Advisory Board for 1972. Pp. 36. (Dublin: National Drugs Advisory Board, 1973.) 25p. [296]

Smith Kline and French Foundation. Tenth Annual Report, 1972. Pp. 8. (Welwyn Garden City, Herts: Smith Kline and French Foundation, 1973.) [27]

The Lister Institute of Preventive Medicine. Report of the Governing Body 1973. Pp. 49. (London: The Lister Institute of Preventive Medicine, Chelsea Bridge Road, 1973.) [27]

Other Countries

Académie Royale de Belgique. Annuaire 1973. Pp. 334. (Bruxelles: Académie Royale de Belgique, 1973.) [256]

World Health Organization. Application of the International Classification of Diseases to Dentistry and Stomatology (ICD-DA). Pp. 114. (Geneva: WHO; London: HMSO, 1973.) 16 Sw. francs; £2; \$4.80. [256]

Beyond Conflict or Compromise: Human Progress, Environmental Protection and the United Nations Development Programme. Pp. 38. (New York: UNDP, United Nations, 1973.) \$2. [256]

Publications du Centre National pour l'Exploitation des Océans. Serie: Rapport Scientifiques et Techniques. No. 14: l'Exploitation de la Coquille Saint-Jacques au Japon. Par Arnaud Muller-Feuga et Joel Quereilou. Pp. 85. (Paris: Centre National pour l'Exploitation des Océans, 1973.) [256]

Science Council of Canada. Report No. 20: Canada, Science and International Affairs. Pp. 66. (Ottawa: Information Canada, 1973.) \$1.25. [256]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Fisheries and Oceanography, 1970/1971. Pp. 69. (Cronulla, NSW: CSIRO, 1973.) [266]

United Nations: Centre for Economic and Social Information. Youth and Population. (Report of the Working Party on Youth and Population, Turin, 17-21 July 1972.) Pp. 48. (New York: United Nations, 1973.) [276]

Unesco. Monographs on Oceanographic Methodology No. 3: A Guide to the Measurement of Marine Primary Production Under Some Special Conditions. Pp. 73. (Paris: Unesco, 1973.) 12 francs; £1; \$3. [276]

National Geographic Society. Research Reports: Abstracts and Reviews of Research and Exploration authorized under Grants from the National Geographic Society during the year 1966. Edited by Paul H. Oehser, under the direction of the Committee for Research and Exploration. Pp. x+321. (Washington, DC: National Geographic Society, 1973.) [296]

Smithsonian Contributions to Zoology. No. 125: Behavior and Ecology of the Asiatic Elephant in Southeastern Ceylon. By George M. McKay. Pp. iv+113. (Washington, DC: Smithsonian Institution Press, 1973. For sale by US Government Printing Office.) \$1.75. [296]

United States Department of the Interior: Geological Survey. Professional Paper 789: Geochemistry of Lower Eocene Sandstones in the Rocky Mountain Region. By James D. Vine and Elizabeth B. Tourtelot. With a section on Direct-Reader Spectrometric Analyses by Raymond G. Havens and Alfred T. Myers. Pp. iv+36. 55 cents. Water-Supply Paper 1873-D: Measurement of Salt-Wedge Excursion Distance in the Duwamish River Estuary, by Means of the Dissolved-Oxygen Gradient. By W. A. Dawson and L. J. Tiller. Pp. iii+27. 25 cents. (Washington, DC: Government Printing Office, 1972 and 1973.) [296]

NORDITA: Nordisk Institut for Teoretisk Atomfysik. Virksomhedsberetning Juli 1971-December 1972. (NORDITA Report.) Pp. 38. (Copenhagen: NORDITA, 1973.) [296]

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